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(54) Title: OLIGONUCLEOSIDE COMPOUNDS AND METHODS FOR INHIBITING TUMOR GROWTH, INVASION AND METASTASIS			
(57) Abstract Oligonucleoside compounds useful in inhibiting expression of focal adhesion kinase protein in animals, and related methods and formulations for reducing cancer cell growth, invasion and metastasis. The compounds are selected to be complementary to a target region of a focal adhesion kinase nucleic acid sequence, preferably human FAK mRNA.			

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cells on a fibronectin-coated substrata, increased phosphorylation of p125^{FAK} has been demonstrated. Kornberg, L., et al., J. Biol. Chem. 267:23439-442 (1992).

Specific integrin expression patterns have been associated with both cellular proliferation and metastasis. For example, overexpression of the $\alpha 5 \beta 1$ integrin in human colon cancer cells has markedly reduced tumorigenicity in nude mice. Varner, J.A., et al., Mol. Biol. Cell 3:232A (1992). In contrast, other integrin expression patterns have been associated with invasion and metastasis, rather than cellular growth. Transfection of the $\alpha 2 \beta 1$ integrin into the RD rhabdomyosarcoma cells has markedly increased tumor metastases in nude mouse tail vein injection assays. Chan, B.M.C., et al., Science 251:1600-1602 (1991). Furthermore, expression of either the $\alpha 6 \beta 4$ laminin receptor or the $\alpha v \beta 3$ integrin has been associated with metastatic behavior in studies of melanoma metastases. Ruiz, P., et al., Cell Adhesion Commun. 1:67-81 (1993), Gehlsen, K.R., et al., Clin. Exp. Metastasis 10:111-120 (1992). These findings further raise the possibility of a significant role for FAK in the metastatic process.

The final property of FAK which also suggests a link to cellular growth is its relationship to the growth stimulation of neuropeptides such as bombesin, vasopressin, and endothelin. These molecules exert mitogenic stimuli via receptors which are coupled to effectors via heterotrimeric G proteins. Stimulation of Swiss 3T3 cells with these neuropeptides has led to a rapid increase in specific p125^{FAK} phosphorylation, suggesting that the effector molecules exert their stimuli via FAK. Zachary, I., et al., J. Biol. Chem. 267:19031-34 (1993). Thus, FAK appears to be a convergent pathway for growth stimulatory neuropeptides, transformation by the v-src oncogene, and integrin-mediated signaling. Zachary, I. & Rozengurt, E., Cell 71:891-894 (1992).

SUMMARY OF THE INVENTION

The present invention relates to compositions and methods for inhibiting the growth, invasion and/or metastasis of tumors or cancer cells using antisense oligonucleoside compounds that are complementary to a portion of an FAK mRNA, preferably human FAK mRNA. The inventors have established that such antisense compounds are effective in inhibiting the expression of the FAK protein product in transformed (i.e. cancerous) human cells, and that such inhibition results in reduced cancer cell growth and adhesion, induction of cell apoptosis, reduced cell motility and invasiveness, reduced cell colony formation and anchorage-independent cell growth, and reduced rates of tumor formation.

The FAK antisense oligonucleoside compounds of the invention are chosen to have a length sufficient to bind to and inhibit the expression of the targeted FAK mRNA. The compounds may be of any suitable length, although typically they will have a sequence of from about 6 to about 40, and preferably about 12 to about 30, linked nucleosides. The nucleoside sequence is chosen to be complementary to a selected FAK mRNA target region sequence, such that the antisense compounds are capable of hybridizing to the selected FAK target region of the FAK mRNA within the subject cells and effecting inhibition of FAK expression. The individual nucleosides of the antisense compounds are linked by internucleoside bonding groups ("backbone" linkages) preferably chosen to afford the compounds stability against degradation by endogenous cellular nucleases, and also to enhance stable and specific hybridization to the target FAK mRNA. Such linkages may include natural phosphodiester linkages, but preferably will include one or more nuclease-resistant non-phosphodiester linkages such as phosphorotriphosphate, phosphorodithioate, alkyl- or arylphosphonate, phosphoramidate, phosphotriester, alkyl- or arylphosphonothioate, aminoalkylphosphonate, aminoalkylphosphonothioate, phosphorofluoridate,

boranophosphate, silyl, formacetal, thioformacetal, morpholino or peptide-based linkages. Specificity and binding affinity toward the target FAK mRNA may be increased through the use of chirally-selected asymmetric linkages, preferably Rp-chiral linkages.

5 The present antisense compounds may be constructed to achieve inhibition of FAK expression by a variety of different mechanisms. For example, the compounds may be designed to form a stable duplex with the RNA so as to block transcription at the ribosome. The duplex blocking mechanism is particularly usefully employed when targeting the 5'-untranslated portion or other
10 non-coding regions of the target mRNA, or elsewhere in the mRNA if ribosomal displacement of the antisense compound does not occur to a significant extent. For target regions where ribosomal displacement is a consideration (e.g., in coding regions), increased duplex stability may be achieved by incorporating a cross-linking moiety in the antisense compound so as to link the hybridized
15 antisense compound to the target mRNA. Alternatively, inhibition of FAK expression may be achieved by using antisense structures which disrupt the integrity or structure of the FAK mRNA molecule, as for example by mRNA cleavage. Cleavage of the target FAK mRNA may be accomplished by choosing antisense sequences capable of activating cellular RNASE H or other endoge-
20 nous cleavage agents, by incorporating a cleavage moiety in the antisense compound, or by co-administering a cleavage substance.

 The FAK antisense oligonucleoside compounds of the invention may also be usefully derivatized or conjugated with, for example, 2'-sugar substituents, particularly electron-withdrawing groups which increase binding affinity;
25 cellular-uptake or membrane-disruption moieties; intercalating agents; radical generators; alkylating agents; detectable labels; chelators; or the like.

 The present invention further includes formulations comprising FAK antisense compounds for use in mammalian cancer therapy, and methods for

using the same. The antisense compounds of the invention are also useful in the *in vitro* or *ex vivo* study of the biological properties of cancer and other mammalian cells, for example in studies of cell growth, invasion, and metastasis, and studies of the inhibition of such properties.

5 These and other aspects of the present invention are described in more detail in the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

10 FIGS. 1A-1B show the amino acid sequences for human (HUMFAK), mouse (MUSFAK) and chicken (CHKFAK) focal adhesion kinase proteins, aligned to show sequence homology.

 FIG. 2 is a Western blot analysis of p125^{FAK} expression in RD (lane 1), BT20 (lane 2), HT29 (lane 3) and C8161 (lane 4) cell lines.

15 FIG. 3 is a Western blot analysis showing progressive p125^{FAK} expression in 23 paired colorectal tumors as the tumors invade and metastasize, wherein paired samples from individual patients are indicated by letters at the bottom.

 FIG. 4 is a Western blot analysis (top) and a graph (bottom) showing the time course of p125^{FAK} expression in C8161 cells exposed to an FAK antisense compound of the invention and to a missense control.

20 FIG. 5 is a Western blot analysis showing specific attenuation of p125^{FAK} expression in RD cells treated with FAK antisense compounds FAK1AS (lane 1) and FAK2AS (lane 2), as well as control samples treated with an equivalent concentration of nonsense compound WNT (lane 3) or with 0.3% lipofectin (lane 4), and untreated control cells (lane 5).

25 FIG. 6A is a bar graph (left) showing loss of cell adhesion in C8161 cells treated with an FAK antisense compound of the invention as compared to control samples treated with a 5bp missense compound (MSN2), and a depiction of stained adherent cells (right) obtained 24 hours after treatment. FIG. 6B is a bar

graph showing percentage loss of cell adhesion in RD cells treated with AS1, AS2, MSN1, MSN2 oligonucleosides, antisense oligonucleosides to WNT and (G)4 oligonucleoside following a 24 hour treatment period. FIG. 6C is a bar graph showing the relative expression of p125^{FAK} in RD cells treated for 24 hours with AS2, MSN2, WNT and (G)4.

FIG. 7 is a Western blot analysis of p125^{FAK} expression in cells treated with either AS2 or MSN2 oligonucleoside for 24 hours. Subsequently, p125^{FAK} expression was analyzed with AS2 treated cells separated into adherent and nonadherent populations; MSN2 treated cells were treated as a whole population. Lane 1 represents parental cells; Lane 2 represents whole population cells treated with MSN2; and Lanes 3 and 4 represent nonadherent and adherent cells, respectively, treated with AS2.

FIGS. 8A-8C are graphs depicting flow-cytometric analyses of C8161 suspension (8A) and adherent (8B) cells treated with a FAK antisense compound of the invention, or with a 5bp missense compound (8C).

FIGS. 9A and 9B are graphs depicting flow-cytometric analyses of RD cells treated with AS2 oligonucleoside for 24 hours. FIG. 9A is a DNA histogram for nonadherent RD cells treated with AS2; FIG. 9B is a DNA histogram for adherent RD cells treated with AS2. FIG. 9C is a 1.2% agarose gel depicting the degree of DNA fragmentation in adherent (lane 1) and nonadherent (lane 2) RD cells treated with AS2.

FIG. 10 are electron micrographs of nonadherent cells treated with AS2 oligonucleoside, showing nuclear condensation and margination of chromatin (10A), apoptotic bodies containing nuclear fragments with sharply delineated masses of compacted chromatin (10B) and shrinkage of cell size and deeply Giemsa stained nuclei.

FIG. 11 is a micrograph (400x magnification) showing apoptosis in tumor cells treated with AS2 or MSN2 oligonucleoside.

FIG. 12 is a bar graph (left) showing inhibition of C8161 cell invasive potential by an antisense compound of the invention compared to a 5bp missense control, and depictions of stained cell filters (right).

FIG. 13 is a graph of mean tumor volume over time, showing reduced tumor growth in athymic nude mice receiving cancer cells treated with an FAK antisense compound of the invention as compared to cells treated with a 5bp missense compound.

DETAILED DESCRIPTION

10 A. FAK Antisense Compounds of the Invention

FIGURE 1 depicts an amino acid sequence for human FAK (labeled "HUMFAK", SEQ ID NO:1), as derived from a cDNA clone isolated from the human breast cancer cell line BT-20. See Example 2 below. This sequence represents 1052 amino acids of the human FAK sequence. The amino acid sequences for mouse ("MUSFAK", SEQ ID NO:2) and chicken ("CHKFAK", SEQ ID NO:3) FAK are also shown. The kinase domain of the respective molecules is boxed. The underlined "recombinant peptide" portion of HUMFAK corresponds to a 66-amino acid region (198 bp) that was subcloned and expressed as a fusion product and used for generation of polyclonal antibodies specific for human FAK (see Example 3 below).

FIGURE 1 also shows two regions, labeled "FAK1AS" and "FAK2AS", which correspond to two FAK mRNA regions targeted for inhibition by complementary antisense oligonucleosides according to the present invention. As detailed in Examples 5A-5H below, such antisense compounds were shown to be effective in inhibiting cancer cell growth, cell adhesion, cell invasion, colony formation and tumor formation, and were effective in inducing cell apoptosis.

The FAK antisense compounds of the invention generally include a sequence of nucleosides that is chosen to be complementary to a target region of the target FAK nucleic acid strand, and particularly the human FAK mRNA strand, such that the antisense compound is capable of hybridizing to the target FAK nucleic acid and inhibiting expression thereof. The term "oligonucleoside" refers to a sequence of nucleoside units linked by internucleoside bonding groups ("backbone" linkages), and thus includes oligonucleotides (linked by phosphodiester backbone linkages) as well as nucleoside polymers linked by structures other than phosphodiester bonds. The term "complementary" refers to a sequence of oligonucleosides (or the individual nucleoside units therein), which is capable of forming hydrogen bonds, and thereby base pairing or hybridizing, with the base sequence of a target region of the target FAK nucleic acid to form a Watson-Crick or "double helix" type structure (whether or not actually helicized) or a portion thereof. Complementary sequences include those which have exact base-by-base complementarity to the target region of the target nucleic acid strand, and also includes oligonucleoside sequences which may lack a complement for one or more nucleotides in the target region, but which still have sufficient binding affinity for the target FAK sequence to form a hybridized structure within the subject (e.g., *in vivo* or intracellular) environment, so as to specifically recognize the target sequence and inhibit expression thereof. Complementary sequences also embrace oligonucleoside compounds, or pairs of distinct oligonucleoside compounds, which have sufficient complementarity to achieve triple-strand binding with a target FAK nucleic acid single-strand sequence, or with a double-strand portion of the target nucleic acid such as a hairpin loop structure, thereby to inhibit FAK expression in the subject environment.

The target FAK nucleic acid sequence is preferably FAK mRNA, including FAK pre-mRNA. The particular target region may be chosen from a

variety of locations in the coding or non-coding portions of the mRNA molecule. Suitable non-coding regions include the 5'-untranslated region, the initiation codon region, the 5'-cap site region, splice acceptor or donor sites, intron branch sites, or polyadenylation regions. Where the target region is a non-coding region, inhibition of protein production can be achieved prior to the translation process by suitable hybridization of the antisense oligonucleoside, and ribosomal displacement of the hybridized oligonucleoside generally does not occur during attempted translation. In such cases translation may be blocked by the effect of complementary hybridization alone, and it will generally not be necessary to incorporate additional inhibition structures (e.g., cross-linking or cleavage moieties) into the antisense compound. Pre-mRNA splicing as a target for antisense oligonucleosides is discussed in R. Kole et al., *Advanced Drug Delivery Reviews*, 6:271-286 (1991). Where the target region is in the coding portion of the FAK mRNA, it is believed that ribosomal displacement of the antisense compound may sometimes occur during the translation process. In such instances it is useful to incorporate cross-linking, cleavage, RNASE H activating or other expression inhibition structures into the antisense compound in order to increase efficacy. Such structures are described in more detail below. The target region, and the associated sequence of complementary nucleosides in the antisense compound, should be selected such that hybridization is specific to the intended FAK target, thus avoiding or minimizing hybridization with non-FAK nucleic acid sequences in the genome of the subject cell or animal that are not intended to be inhibited. In this regard, publicly-available computer listings of gene sequences may be checked so as to avoid the selection of FAK target sequences similar to known non-FAK genes.

The FAK antisense oligonucleosides of the present invention may be of any suitable length, but preferably are between about 6 to about 40 nucleosides in length, and more preferably between about 12 to about 30 nucleosides. The

length of a particular antisense compound, the number of complementary bases in the compound, and the identity and location of the complementary bases may be adapted so that suitable target specificity and binding affinity will be achieved under the conditions in which the compound will be used. These conditions include, for example, the effective concentration of the antisense compound inside the cell, the concentration and turnover rate of the target sequence, the desired level of reduction of concentration of the target sequence, the efficacy of expression inhibition, and the mode of inhibition (e.g., catalytic or non-catalytic).

The present FAK antisense compounds preferably are modified to render them resistant to degradation by cellular nucleases or other enzymes that are present *in vivo*. This modification can be accomplished by methods known in the art, e.g., by incorporating one or more internal artificial internucleoside linkages (such as by modifying the phosphodiester linkage to include alternate or additional groups in conjunction with a phosphorus atom, e.g., by replacing one of the non-bridging phosphate oxygens in the linkage with sulfur, methyl or other atoms or groups), and/or by blocking the 3' end of the oligonucleoside with a capping structure. Preferred examples of such nuclease-resistant non-phosphodiester linkages include phosphorothioate, phosphorodithioate, alkyl- (especially methyl-) and arylphosphonate, phosphoramidate, phosphotriester, alkyl- (especially methyl-) and arylphosphonothioate, aminoalkylphosphonate, aminoalkylphosphonothioate, phosphorofluoridate, boranophosphate, silyl, formacetal, thioformacetal, morpholino and peptide-based linkages. Mixtures of such linkages, including mixtures with one or more phosphodiester linkages, are likewise useful and can be utilized to adjust the binding affinity, specificity and expression inhibition characteristics of the subject compounds while maintaining a suitable level of nuclease resistance.

Synthetic methodologies for preparing antisense compounds containing such backbone linkages are known in the art. For example, commercial

machines, reagents and protocols are available for the synthesis of oligonucleosides having phosphodiester and certain other phosphorus-containing internucleoside linkages. See, for example, Gait, M.J., Oligonucleotide Synthesis: A Practical Approach (IRL Press, 1984); Cohen, Jack S., Oligodeoxynucleotides Anti-sense Inhibitors of Gene Expression (CRC Press, Boca Raton FL, 1989); and Oligonucleotides and Analogues: A Practical Approach (F. Eckstein, 1991); Agrawal, S. (ed.), Protocols for Oligonucleosides and Analogs. Methods in Molecular Biology, Vol. 20 (Humana Press, Totowa N.J. 1993). Synthetic methods for preparing methylphosphonate oligonucleosides are described in Agrawal, above, Chapter 7, pages 143-164 (Hogrefe, R.I.), and in PCT Application Nos. WO 92/07864 and WO 92/07882. Preparation of oligonucleosides having various non-phosphorus-containing internucleoside linkages (such as morpholino, formacetal and peptide nucleic acid (PNA) linkages and the like) is described in, for example, United States Patent No. 5,142,047 and in PCT Publication Nos. WO 92/02532 (Reynolds, M.A., et al.) and WO 93/13121 (Cook, P.D.). The disclosures of these synthetic methodology references are incorporated herein by reference.

Where it is desired to use an antisense compound that is capable of activating RNASE H for cleavage of the target FAK nucleic acid, a number of other structural considerations come into play. First, it has been reported that uncharged backbone linkages are incapable of activating RNASE H. As a result, such antisense compounds should include an RNASE H activating portion comprising at least about three consecutive charged (anionic) internucleoside linkages, as for example phosphodiester, phosphorothioate or phosphorodithioate linkages or mixtures thereof. Second, it has been reported that various 2'-sugar substituents (particularly electron-withdrawing groups such as 2'-O-alkyl or 2'-fluoro) will render the substituted portion of the antisense strand non-activating to RNASE H, even though binding affinity toward the target nucleic acid is

increased. Inoue, H., et al., FEBS Letters 215:327-330 (1987); Monia, B.P., et al., J. Biol. Chem. 268:14514-522 (1993). Accordingly, the charged-backbone RNASE H activating portion of such compounds should be 2'-unsubstituted, although 2'-substituents may usefully be employed in other (particularly terminal) non-activating portions of the compound to increase binding affinity. Third, in order to increase nuclease resistance in such antisense compounds, it is preferred to incorporate non-phosphodiester backbone linkages, as for example methylphosphonate, phosphorothioate or phosphorodithioate linkages or mixtures thereof, into one or more non-RNASE H-activating regions of the compounds. Such non-activating regions may additionally include 2'-substituents as discussed above, and, as discussed below, may include chirally-selected backbone linkages in order to increase binding affinity and duplex stability.

Other functional groups may also be joined to the oligonucleoside sequence to instill a variety of desirable properties, such as to enhance uptake of the oligonucleoside sequence through cellular membranes, to enhance stability or to enhance the formation of hybrids with the target nucleic acid, or to promote cross-linking with the target (as with a psoralen photo-cross-linking substituent). See, for example, PCT Publication No. WO 92/02532. Examples of cellular-uptake or membrane-disruption moieties include polyamines, e.g. spermidine or spermine groups, or polylysines; lipids and lipophilic groups; polymyxin or polymyxin-derived peptides; octapeptin; membrane pore-forming peptides; ionophores; protamine; aminoglycosides; polyenes; and the like. Other potentially useful functional groups include intercalating agents; radical generators; alkylating agents; detectable labels; chelators; or the like.

Where it is desired to effect cleavage of the target FAK nucleic acid strand with the antisense compound, a suitable cleavage moiety may be incorporated into the compound. Such cleavage moieties preferably include functional groups

selected to achieve one or more of the functions associated with enzymatic cleavage of RNA. These functions include (1) providing a nucleophilic moiety for attack on the target phosphorus atom, especially by deprotonation of the 2'-OH hydrogen of a target sugar in the target region of the FAK RNA (as achieved, for example, by increasing the local pH about the target sugar and/or by providing a basic or nucleophilic moiety in the vicinity of the target sugar); (2) supplying a proton or other electrophilic moiety for interaction with a phosphorus-bonded lone oxygen atom of the target RNA to form, for example, a protonated phosphate diester (as achieved, for example, by operation of an acidic or electrophilic moiety of the cleavage compound); (3) stabilizing the cleavage transition state, i.e., providing a structure on the cleavage compound to stabilize the intermediate structure or structures assumed by the target RNA during the cleavage mechanism, as by the inclusion of an acid-base moiety and/or other moieties which afford charge neutralization or hydrogen bonding stabilization to the intermediate (particularly polyfunctional groups capable of stabilizing a dianionic phosphorane in a trigonal bipyramidal configuration); and (4) providing a structure to protonate the leaving 5'-O oxygen atom of the target site, as by operation of an acidic moiety of the cleavage compound. See generally Jubian, et al., J. Am. Chem. Soc. 114:1120-1121 (1992), which is incorporated by reference. Preferably, the cleavage moiety comprises two or more distinct functional groups selected to provide two or more of the functions of proton donation, proton acceptance, hydrogen bonding and charge neutralization. Among these are cleavage moieties comprising two or more amino groups, and wherein at least one amino group is substantially protonated, and at least one amino group is substantially nonprotonated, at physiological pH. Additionally, or alternatively, the cleavage compounds may include a strong Lewis acid moiety, as for example a chelated metal species, which activates the phosphorus-oxygen center of a target phosphodiester bond (or of a target pyrophosphate

linkage in the case of a 5'-cap region of a target RNA sequence) for direct hydrolytic cleavage by *in situ* water or hydroxide ion. In addition, such antisense cleavage compounds will preferably include a substituent or portion that facilitates rotation of a target RNA sugar portion about the phosphodiester backbone of the target RNA, preferably to position a 2'-OH group of the target RNA for in-line, intramolecular attack on a neighboring phosphorus atom of the target backbone (as achieved, for example, by incorporating an intercalating moiety, a base-omission mismatch, or some other non-complementary structure within the cleavage compound).

Oligonucleosides having one or more chirally pure internucleosidyl linkages (particularly Rp-chiral linkages) may be used and may be preferred in order to increase binding affinity between the subject antisense compounds and the target FAK nucleic acid sequence. Such oligonucleosides, for example with methylphosphonate or phosphorothioate linkages, may be prepared using methods as those described in Lesnikowski, et al., *Nucleic Acids Research* 18(8):2109-2115 (1990), Stec, et al., *Nucleic Acids Research* 19(21):5883-5888 (1991), Cook, U.S. Patent No. 5,212,295, or PCT Publication No. WO 93/08296 (Hoke, G.D. & Cook, P.D.). These references are likewise incorporated by reference herein.

The FAK antisense compounds for use in the instant invention may be administered singly, or tandem or separate combinations of the compounds may be administered for adjacent or non-neighboring targets or for combined effects of anti-sense mechanisms in accordance with the foregoing general mechanisms. For example, two separate tandem antisense compounds having complementarity to neighboring target subregions in the FAK nucleic acid strand may be used, where one of the tandem compounds provides a cleavage moiety and the other tandem compound provides a non-complementary structure as described above. Alternatively, each of the two tandem compounds may provide some portion of

an RNASE H activating region, or some portion of a cleavage moiety, whereby the two compounds act cooperatively following hybridization to adjacent regions in the target strand to effect cleavage or other inhibition of expression of the target strand. Such tandem compounds would be expected to provide greater target specificity (and decreased inhibition of unintended nucleic acid sequences) inasmuch as separate hybridization of two separate antisense compounds is required to achieve inhibition.

B. Methods and Therapeutic Compositions

When used in mammalian therapy, the FAK antisense compounds may be administered in any convenient vehicle that is physiologically acceptable. The compounds can be formulated for a variety of modes of administration, including systemic, topical or localized administration. Techniques and formulations generally may be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, latest edition. In each case, a therapeutically effective amount of the antisense compound is administered in order to prevent or inhibit the translation of the target FAK nucleic acid. The antisense compound is generally combined with a carrier such as a diluent or excipient which may include fillers, extenders, binding, wetting agents, disintegrants, surface-active agents, or lubricants, depending on the nature of the mode of administration and dosage forms. Typical dosage forms include tablets, powders, liquid preparations including suspensions, emulsions and solutions, granules, capsules and suppositories, as well as liquid preparations for injections.

In the pharmaceutical formulation the antisense compound may be contained within a lipid particle or vesicle, such as a liposome or microcrystal, which may be suitable for parenteral administration. The particles may be of any suitable structure, such as unilamellar or plurilamellar, so long as the antisense oligonucleotide is contained therein. Positively charged lipids such as N-[1-(2,3-

diolcoyloxi)propyl]-N,N,N-trimethyl-ammoniummethylsulfate, or "DOTAP," are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well known. See, e.g., U.S. Patents Nos. 4,880,635 to Janoff et al.; 4,906,477 to Kurono et al.; 4,911,928 to Wallach; 4,917,951 to Wallach; 5 4,920,016 to Allen et al.; and 4,921,757 to Wheatley et al. Other non-toxic lipid based vehicle components may likewise be utilized to facilitate uptake of the antisense compound by the cell.

For systemic administration, injection may be preferred, including intraarterial, intravenous and intraperitoneal injection (which are especially 10 preferred), as well as intramuscular and subcutaneous injection. For injection, the cleavage compounds of the invention are formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are 15 also included. In some instances, the compositions may be infused upstream from the site of the cells whose activity is to be modulated. Implantable drug pumps, as for example Infusaid® pumps (Infusaid, Inc.), are useful for delayed-release intraarterial administration.

Systemic administration can also be by transmucosal or transdermal 20 means, or the compounds can be administered orally. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, bile salts and fusidic acid derivatives for transmucosal administration. In addition, detergents may be used to facilitate permeation. 25 Transmucosal administration may be through use of nasal sprays, for example, as well as formulations suitable for administration by inhalation, or suppositories. For oral administration, the oligonucleosides are formulated into conventional

as well as delayed release oral administration forms such as capsules, tablets, and tonics.

Antisense compounds of the invention may also be administered by introducing into the cell a DNA construct which produces an antisense compound as described herein within the cells. Such a DNA construct typically
5 contains, in operable association with one another, a transcriptional promoter segment operable in the target cell, a DNA segment that encodes the antisense compound, and a transcription termination segment. Such DNA constructs may be provided in a pharmaceutical formulation as described herein. Such DNA
10 constructs are made and used in accordance with known techniques as set forth in M. Inouye, U.S. Patent No. 5,190,931, the disclosure of which is incorporated by reference herein in its entirety.

For topical administration, the oligonucleosides for use in the invention are formulated into ointments, salves, gels, or creams, as is generally known in
15 the art.

The localized concentration or amount administered to an animal subject may be determined empirically and will depend upon the purpose of the administration, the area to be treated, the effectiveness of the composition, and the manner of administration. The localized concentration at the site of the
20 targeted cells will desirably be in the range of about 0.05 to 50 μ M, or more particularly 0.2 to 5 μ M, although higher or lower dosages may be employed as appropriate. In particular, it is contemplated that relatively high dosage levels may safely be employed in the present context because the FAK gene is overexpressed in cancer cells, and is expressed at relatively low levels in non-
25 cancerous cells. For administration to a subject such as a human, a dosage of from about .01, .1, or 1 mg/kg up to 50, 100, or 150 mg/kg or more may typically be employed.

The present compounds may also be used in *in vitro*, *ex vivo* or in other non-therapeutic modes in order to study the biological properties of the FAK gene and protein, and their role in normal or cancer cell development, propagation, migration and the like. The present invention is also useful *in vitro* in tissue culture and fermentation techniques where it is desired to inhibit or reduce cell adhesion to facilitate growth of the cells, subsequent processing of the cells, production of proteins or other compounds from the cells, etc. Other uses of the present invention, and suitable antisense compounds to achieve the goals of the invention, will be apparent to those skilled in the art in view of the present disclosure, including the examples that follow. However, it will be understood that the specific examples herein, and the specific antisense structures described, while useful in appreciating the utility of the invention, are not intended to limit the scope of the invention as claimed hereinafter.

15 C. Examples

Example 1. Preliminary Studies -- Isolation of Human FAK Homolog and Measurements of Cellular Expression

In preliminary studies relating to the present invention, homologous tyrosine kinase gene fragments were cloned from human cell lines and primary human tumors using low stringency PCR amplification and degenerate oligonucleotide primers based on catalytic domain consensus sequences common to all tyrosine kinases. Cance, W.G., et al., Int. J. Cancer 54:571-577 (1993) (incorporated by reference herein). Using these methods, a 210bp gene fragment of the human homolog of FAK was isolated from a primary human sarcoma, and was found to be expressed in sarcoma, breast and colon cell lines. Weiner, T.M., et al., Ann. Surg. Oncol. 1:18-27 (1994) (incorporated by reference herein). The expression of FAK in normal, adenomatous, invasive, and metastatic human tissue was also studied. Using Northern analysis, increased levels of FAK were

found in 1 of 8 adenomatous tissues, in 17 of 20 invasive tumors, and in all 15 metastatic tumors. Paired samples of normal tissue did not express detectable FAK mRNA. See Table 1. This association of FAK overexpression with invasion and metastasis was a finding common to both the epithelial and mesenchymal tumors analyzed. Furthermore, upon comparison of the levels of FAK mRNA in paired samples from colon cancer patients, a progressive increase in densitometrically indexed FAK mRNA was found in 3 of 4 samples as the tumor invaded and metastasized. Weiner, T.W., et al., The Lancet 342:1024-1025 (1993) (incorporated by reference herein). These studies are believed to have established the first translational link of FAK expression to the progression of human cancer.

TABLE 1. SUMMARY OF NORTHERN ANALYSES OF FAK IN HUMAN TUMORS

TISSUE:	<u>NORMAL</u>	<u>BENIGN</u>	<u>PRIMARY</u>	<u>METASTASIS</u>
Colon	0/4	1/6	7/8	7/7
Breast		0/2	9/11	4/4
Other*	0/2		1/1	4/4
Total	0/6(0%)	1/8(12%)	17/20(85%)	15/15(100%)

*Normal muscle (2), Primary Thyroid Carcinoma (1) with paired nodal metastasis (1), Metastatic Carcinoid (1), Squamous Cell Carcinoma (1) and Melanoma (1)

Example 2. Identification and Characterization of a Human FAK cDNA Clone

The 210bp FAK gene fragment described above was used as a probe to isolate larger cDNA clones. A cDNA library was first constructed from the BT-20 human breast cancer cell line. Poly-A⁺ RNA was isolated from BT20 cells, and first strand synthesis was carried out using a poly-T primer and Maloney-

Murine Leukemia Virus Reverse Transcriptase. Synthesis of the second strand was performed using DNA Polymerase I, followed by ligation of Not1 linker adapters, Not1 restriction endonuclease digestion, and ligation of the cDNA fragments into a Not1 digested cloning vector. A cDNA clone, spanning 1052 amino acids of the predicted sequence, was identified (see FIG. 1, "HUMFAK").

The clone was found to be homologous to both the mouse and chicken FAK sequences and identical to a recently-published human cDNA clone derived from T-cells (Whitney, G.S., et al., DNA Cell Biology 12:823-830 (1993)).

Example 3. Generation of Recombinant FAK Polypeptide and Anti-FAK Polyclonal Antibodies

A 198bp segment of the FAK cDNA clone of the preceding example was subcloned into a pQE expression vector as described in Bujard, H., et al., Methods in Enzymology 155:416-433 (1987). Fusion protein expression was induced by IPTG at 37°C, followed by purification under denaturing conditions on a Ni-NTA resin column. This provided a hexahistidine fusion protein containing an amino-terminal 6kD fragment of the FAK clone. This segment of the FAK protein (see FIG. 1) was selected in order to allow generation of FAK-specific polyclonal antibodies which would not cross-react with the carboxy-terminal 41kD FAK-related non-kinase protein (FRNK, see Schaller, M.D., et al., Mol. Cell. Biol. 13:789-791 (1993)). The purified fusion protein was analyzed by SDS/PAGE, excised from the gel and injected into rabbits to prepare polyvalent sera.

The antisera recognized a 125kD protein by Western blotting against cell lines (C8161, RD, BT20 and BT474) known to overexpress FAK. The rhabdomyosarcoma (RD) cell line was grown in either RPMI-1640 with 10% heat-inactivated fetal calf serum (FCS), penicillin (100 units/ml) and streptomycin (100 mg/ml) or Dulbecco's Modified Eagle's Medium (DMEM-H)

supplemented with 10% fetal bovine serum (FBS, Hyclone). The C8161, BT20 and BT474 cell lines were grown in RPMI-1640, and maintained at 37°C in a 5% CO₂ incubator. In the case of the BT474 cell line (purchased from ATCC in Rockville, Maryland), the RPMI-1640 medium was supplemented with 10% FBS, 10 µl/ml insulin and 300 µg/ml L-glutamine. Antibody characterization further included titering studies to a 1/5000 dilution along with blocking experiments. Antibody reactivity was completely inhibited by the addition of recombinant 6kD blocking peptide. Blocking was accomplished by preincubation of the titrated antisera with progressive levels of the recombinant antigenic peptide prior to Western analysis until complete attenuation of the 125kD signal.

Example 4. Protein-level Measurement of FAK Overexpression in Normal and Cancerous Tissue and Cell Lines

The expression of p125^{FAK} was measured in a variety of normal and cancerous human tissue and cell line samples using the anti-FAK antibody obtained as described above.

Initially, FAK expression in the RD (embryonal rhabdomyosarcoma), BT20 (breast adenocarcinoma), HT29 (colon adenocarcinoma), C8161 (melanoma) and other human tumor cell lines was studied. As shown in FIG. 2, the highest levels of expression occurred in the RD cell line, but expression was also detected in the BT20, HT29 and C8161 lines. In contrast, some of the cell lines (such as the breast cell line SK-BR-3) expressed low levels of p125^{FAK}.

The anti-FAK antibody was also used to assess the change in levels of p125^{FAK} expression in normal, neoplastic, invasive and metaplastic human tissues. In particular, 91 different tissue samples including epithelially-derived colon and breast cancers, as well as mesodermally-derived sarcomas were studied by Western blot analysis. Colon samples included normal mucosa only; benign, non-invasive polyps; invasive polyps; invasive primary cancers and both

liver and peritoneal metastatic specimens. Breast samples included benign, non-invasive fibroadenomas; normal breast tissue paired with the infiltrating ductal lesions and a lymph node metastasis. Sarcoma samples included normal muscle; benign mesenchymal tumors such as lipomas and leiomyomas; invasive sarcomas including leiomyosarcoma, rhabdomyosarcoma, neurofibrosarcoma, liposarcoma, synovial sarcoma and fibrohistiosarcoma. Other specimens studied included normal liver and hypercellular parathyroid lesions.

By way of example, colon tumor samples were obtained through operative specimens via IRB-approved protocols and banked at the Tissue Procurement Facility of the Lineberger Comprehensive Cancer Center. Protein was extracted from snap-frozen primary tissues. A 1 cm³ section of tissue was placed in 3 ml of NP-40 lysis buffer (1% Triton X-100, 20mM Tris, pH 7.4, 150 mM NaCl, 5mM EDTA, 1mM Na₃VO₄, 10mg/ml each of aprotinin and leupeptin). The tissue was homogenized in the lysis buffer using a Polytron (Brinkman), then centrifuged for 15 minutes at 4°C in a microcentrifuge. The amount of protein was measured by the BCA protein assay (Pierce, Rockford, IL). Cell lysate containing 30µg of protein was subjected to 10% SDS/PAGE and electroblotted onto a nitrocellulose membrane as described by Towbin, H., et al., Proc. Natl. Acad. Sci. USA 76:4350-4354 (1979). Immunodetection of blotted p125^{FAK} was accomplished using a 1/2000 titer of anti-FAK antibody along with a 1/5000 titer of anti-rabbit IgG horseradish peroxidase conjugate (Amersham) in non-fat milk. The blots were washed several times in 0.1% TBST (20mM Tris pH 7.4, 150mM NaCl, 0.1% Tween20) and visualization was achieved by chemiluminescence using the ECL detection system (Amersham) followed by X-ray film exposure. In FIG. 3, which shows Western blots for paired colon cancer series, the labels read as follows: NC, normal mucosa; CC, primary invasive tumor; LM, liver metastasis; PM, peritoneal metastasis; RD, embryonal rhabdomyosarcoma cell line (positive control).

These studies demonstrated the existence of progressive up-regulation of FAK from normal to invasive and metastatic phenotypes, consistent with the transcriptional data described above. The results from the 91 tissue samples are summarized in Table 2. In paired normal and neoplastic colon samples there was no FAK expression in 9 normal mucosal specimens compared to overexpression in 6/7 primary invasive tumors and 9/11 metastatic lesions. These results demonstrated progressive increases in p125^{FAK} expression as tumors invade and metastasize. Additionally, five neoplastic, pre-invasive villous adenomas showed high FAK levels, whereas there was no signal in tubular polyp specimens, suggesting FAK overexpression may be an early event as transformed cells become invasive. In a similar measurements of paired breast cancer samples, 9/16 infiltrating ductal lesions demonstrated FAK overexpression with no signal detectable in the matched normal tissue. Finally, analysis of sarcomas, a histologically diverse family of mesenchymal tumors, showed the highest levels of FAK expression in the biologically aggressive, large (>5cm), high grade lesions. In contrast to invasive tumors, hypercellular neoplastic tissues without invasive potential, such as parathyroid adenomas, did not overexpress FAK. Significant levels of p125^{FAK} expression were detected in 3 samples of large, colorectal villous adenomas, perhaps indicating that these tumors were in the process of becoming invasive. It was notable that the only tumor which did not express high levels of p125^{FAK} was a retroperitoneal colorectal cancer recurrence which slowly developed and was resected 4 years after initial colectomy. Thus, these results confirmed our initial observations, linking overexpression of FAK to the invasive and metastatic phenotype, and suggested that more rapidly growing tumors expressed higher levels of p125^{FAK}.

These observations not only demonstrated up-regulation of p125^{FAK} expression as a tumor became invasive and metastatic, but also suggested that

p125^{FAK} overexpression accompanied signaling pathways toward invasion and metastasis for a variety of tumors of both epithelial and mesenchymal origin.

TABLE 2. FAK EXPRESSION IN HUMAN TUMORS

TISSUE:	NORMAL	NEOPLASTIC NON-INVASIVE	PRIMARY INVASIVE	METASTASIS
Colon	0/9	5*/6	6/7	9/11
Breast	0/16	0/2	9/16	1/1
Sarcoma	0/2	0/5	8/8	2/2
Other**	0/2	0/4	-	-
Total	0/29	5/17	23/31	12/14

*Villous adenomas (>2cm)
 **Normal liver (2), Parathyroid adenoma (4)

Example 5. Effect of Antisense Oligonucleosides in Inhibiting FAK Expression and FAK-Related Biological Activities

Antisense oligodeoxyribonucleotides having complementarity to portions of the human FAK mRNA were synthesized in order to study their efficacy in inhibiting FAK expression in human tumor cells and their effect on the FAK-related biological properties of the cells. It was shown that the antisense compounds were not only useful in inhibiting FAK expression, but also inhibited tumor cell growth, cellular adhesion properties, cell motility, cell colony formation, and tumor formation. The compounds were also found to induce cell apoptosis.

30 A. Synthesis of Oligodeoxyribonucleosides

Two separate regions near the 5'-terminus of the human FAK clone were selected for targeting by antisense oligodeoxyribonucleotides (see FIG. 1).

Complementary phosphorothioate-linked antisense oligonucleosides having, respectively, 20 and 24 nucleosides were prepared with the following sequences:

FAK1AS: 5'-ACACTTGAAGCATTCCTTATCAAA-3', SEQ ID NO:4

FAK2AS: 5'-ATAATCCAGCTTGAACCAAG-3', SEQ ID NO:5

5 These sequences have complementarity with the selected target regions of the human FAK mRNA as follows:

HUMFAK ...Phe Asp Lys Glu Cys Phe Lys Cys ...
5' - ...UUU GAU AAG GAA UCG UUC AAG UGU ...-3'
FAK1AS 3' -AAA CTA TTC CTT AGC AAG TTC ACA -5'

HUMFAK ...Leu Gly Ser Ser Trp Ile Ile ...
5' - ...CUU GGU UCA AGC UCG AUU AUU ...-3'
10 FAK2AS 3' -GAA CCA AGT TCG ACC TAA TA-5'

In addition, control sequences having a 2-base or a 5-base mismatches were prepared as follows (mismatched bases are underlined):

MSN1: 5'-ATAATCGAGCTTCAACCAAG-3', SEQ ID NO:6

15 MSN2: 5'-ATAATCGACGTTCAAGCAAG-3', SEQ ID NO:7

A "nonsense" control sequence, derived from the mouse *wnt* protooncogene which was not expressed in the cell lines under study, was also prepared for use in certain of the studies described below:

WNT: 5'-AGCCCGAGCAGGTGGGGCTC-3', SEQ ID NO:8

20 Another control sequence, a 24-mer containing GGGG [(G)4] nucleotide, which has been shown to have aptomeric effects on cell lines with a reduction in cell proliferation, was used in some of the studies described below:

(G)4: 5'-TATGCTGTGCCGGGGTCTTCGGGC-3', SEQ ID NO:9

The specificity of these sequences was confirmed in GeneBank.

25 The oligonucleosides were synthesized using standard phosphoramidite chemistry, in the course of which the internucleoside linkages were converted to phosphorothioate linkages to prevent cellular degradation by RNases. After

synthesis, the oligonucleosides were extracted several times with phenol-chloroform, and then ethanol-precipitated and reconstituted in Hanks' balanced salt solution (HBSS) and frozen at -20°C for storage.

5 B. General Procedures for Application of Antisense Oligonucleosides to Cell Samples

10 In the following studies, the oligonucleosides were first preincubated to a final concentration of 0.15 μ M with 0.3% lipofection reagent (Gibco BRL) in serum-free Opti-Mem medium (Gibco BRL) in order to form a stable lipid-DNA complex for optimizing transfection. Cell samples were typically prepared by seeding approximately 2×10^5 cells in six-well tissue culture plates with 2 ml of medium, and incubating to 60% confluence. As detailed below, the cells studied included melanoma (C8161), embryonal rhabdomyosarcoma (RD), breast adenocarcinoma (BT20) and breast ductal carcinoma (BT474) cells. The growth medium typically comprised 2 ml RPMI-1640 with 10% heat-inactivated fetal calf serum (FCS), penicillin (100 units/ml) and streptomycin (100 μ g/ml). Normal human fibroblast (NHF) cell lines were grown in Eagle's MEM supplemented with 10% FCS.

15 The lipid-DNA solution was applied to the cells under study by gently overlaying a measured portion of the solution onto a 60% confluent monolayer of cells, followed by incubation for a measured time (typically 0-24 hours) at 37°C in a 5% CO₂ incubator. After this time, the antisense oligonucleoside containing medium was typically removed and the suspension cells isolated, washed and resuspended into 2 ml of normal growth medium. As appropriate, 20 the resuspended cells were replaced onto the remaining adherent cell population for further study.

C. Inhibition of p125^{FAK} Expression by FAK Antisense Compounds

Equal numbers of cells were cultured in six-well tissue culture plates and exposed to oligonucleoside/lipofectin reagent solution for varying periods (0-24 hours) as described above. The cells were then allowed to recover for 24 hours. Measurement of p125^{FAK} expression was performed by Western blot analysis with the FAK-specific antibody described above following standard protein level analysis (Pierce).

Attenuation of p125^{FAK} expression in C8161 cells began after 12 hours of exposure to antisense compounds FAK1AS and FAK2AS and was completely abrogated by 18 hours, as exemplified in FIG. 4. Under the same conditions, no significant reduction in p125^{FAK} expression occurred in cells treated with the 5-base mismatch sequence MSN2 or with lipofectin alone. There was a significant attenuation of p125^{FAK} expression with the 2-base mismatch sequence MSN1, although not as complete as with the antisense sequences. FAK expression was seen to recover by 4 days after antisense treatment.

Similar attenuation of FAK expression was obtained when RD cells were treated with the antisense compounds. As shown in FIG. 5, both antisense oligonucleosides completely abrogated p125^{FAK} expression, whereas there was only a minimal reduction in FAK expression in the control samples treated with the WNT oligonucleoside or with lipofectin (0.3%) alone. In addition, the total protein concentrations in the RD cells did not change with FAK attenuation, as indicated by simple Coomassie-stained gels of total protein extracts.

The effects of the antisense oligonucleosides appeared to be highly specific for FAK. Since application of certain oligonucleotide sequences is known to result in general inhibition of gene expression, we examined the expression in antisense treated cells of other tyrosine kinases known to associate with FAK. The expression of p60^{src} and p59^{lyn} were not significantly altered in these cells. These results suggested not only that the antisense effects of FAK

were specific, but also that FAK may function as a downstream element to *src* and *fyn* in these cellular signaling pathways.

D. Inhibition of Cellular Adhesion by FAK Antisense Compounds

5 Disruption of FAK signaling pathway with the FAK antisense oligonucleosides was shown to cause profound changes in cell phenotype. The most significant change observed was a marked loss of cellular adhesion (see FIG. 6A). In each of the model cell lines (C8161, RD, BT20 and BT474), the
10 antisense oligonucleosides appeared to disrupt the cell-matrix interactions. After exposure to antisense, treated cells showed a tendency to round-up and enter suspension. This cellular morphologic effect was progressive throughout the duration of antisense oligonucleoside exposure and continued beyond the twenty-four hour treatment period.

15 By way of example, the effect of the antisense compounds FAK1AS and FAK2AS on cellular adhesion properties was measured by exposing C8161 cells to FAK antisense or to the 5bp missense control MSN2 for 24 hours. The numbers of cells adherent to the cell culture plates (solid bar) and cells in suspension (white bar) were counted at 0, 6, 12, 18 and 24 hours after antisense
20 or control oligonucleosides were added (see FIG. 6A). Loss of adherence in the FAK antisense-treated cells began between 12 and 18 hours after oligonucleoside addition, correlating with the loss of p125^{FAK} expression (FIG. 4). This loss of adhesion was also visualized in hematoxylin and eosin-stained cells 24 hours after FAK antisense treatment. Cells treated with the 5bp missense sequence
25 MSN2 retained their normal adherent characteristics in the staining studies. Similar inhibition of cell adhesion was observed with RD and BT20 cells treated with FAK antisense oligonucleosides. Using RD cells, adherence loss after 24 hours was approximately 40% using AS1 and nearly 70% using AS2 antisense oligonucleoside (FIG. 6B). While 60% of the RD cells lost adherence using

MSN1 oligonucleoside, only about 10% of cells treated with MSN2 lost adherence (see FIG. 6B) following 24 hours of oligonucleoside treatment. To ensure that the phenotypic changes were specifically caused by attenuation of p125^{FAK}, normal human fibroblasts, which did not express high levels of p125^{FAK}, were treated with AS2 oligonucleoside. No significant changes were observed in antisense-treated cells compared to missense-treated cells.

The observed loss of adherence was not specific to sarcoma cells only. When a human epithelial breast cancer cell line (BT474) and a melanoma cell line (C8161) were treated with AS2 oligonucleoside, a marked loss of adherence was also observed in both of these tumor cell lines. Western blot analyses of adherent and nonadherent populations showed that p125^{FAK} was undetectable in nonadherent cells but was not altered in either antisense-treated or missense-treated adherent cells for the RD, C8161 and BT474 cell lines (FIG. 7).

RD cells treated with either of the control oligonucleosides WNT or (G)4 did not show significant loss of adherence (FIG. 6B) and expression of p125^{FAK} was unchanged (FIG. 6C). The loss of adherence following treatment with AS2 was found to be specific to AS2, correlating with the attenuation of p125^{FAK} expression.

20 E. Inhibition of Cell Growth and Inducement of Apoptosis by Attenuation of p125^{FAK} Expression

Apoptosis was evaluated by performing analyses of cell viability, flow cytometry, DNA fragmentation, Giemsa staining and electron microscopic morphology on certain cells following 24 hours of oligonucleoside treatment. Viable cells were counted following staining with 0.4% trypan blue. For Giemsa staining, cells were centrifuged onto microscopic slides using a Cytospin 2 centrifuge (Shandon Lipshaw, PA) and stained with a Diff-Quik stain kit (Baxter). Quantitation of the number of apoptotic cells was accomplished by

counting the number of apoptotic cells versus the number of total cells sighted in ten 400X microscopic fields. For flow cytometric analysis, cells were pelleted, washed in HBSS and fixed with 70% ethanol for 1 hour at 4°C. Cells were then washed in HBSS and resuspended in PI buffer (20 µg/ml propidium iodide, 20 µg/ml RNASE in PBS, pH 7.4) at a final concentration of 1×10^6 cells/ml and analyzed using FACScan (Becton-Dickinson).

Analysis of internucleosomal degradation of genomic DNA was performed following oligonucleoside treatment. The cells were collected from each of the separated adherent and nonadherent populations, rinsed with HBSS and lysed in 100 µl of lysis buffer [10mM TRIS-HCl (pH 8.0), 10mM EDTA (pH 8.0), 0.5% Triton X-100 (Sigma)]. Lysates were centrifuged at $13,000 \times g$ for 20 minutes and supernatants containing soluble fragmented DNA were collected, treated with RNaseA (100 µg/ml) for 1 hour at 37°C, followed by proteinase K (200 µg/ml) treatment in 1% SDS for 2 hours at 50°C. The samples were then extracted twice using phenol-chloroform, once again using chloroform, and then ethanol precipitated with one-tenth volume sodium acetate (pH 5.3) and two volumes ethanol (100%) for 1 hour at -70°C. Electrophoresis of 250 ng DNA was performed on a 1.2% agarose gel in 1 XTAE (0.04 M TRIS-acetate, 1 mM EDTA, pH 8.0) for 1.5 hours at 50V. The gel was then stained in 1 XTAE and 0.5 µg/ml ethidium bromide for 15 minutes at room temperature.

Electron microscopic analysis of cell morphology was performed on treated adherent and nonadherent cells. Nonadherent cells were collected, washed with warm serum-free media, and then fixed using 3% glutaraldehyde in medium overnight at 4°C. Samples were rinsed with PBS, embedded in Epon and cured for 3 days. Thin sections (approximately 60-90 nm) were stained with 5% uranylacetate and 2.7% lead citrate and examined using a Zeiss transmission electron microscope.

Evaluation of nonadherent antisense-treated cells by trypan blue exclusion showed that they were approximately half the size of the adherent cells and exhibited greater than 90% viability, evidencing that the nonadherent cells were not simply necrotic. Flow-cytometric analysis of propidium iodide-stained C8161 suspension cells (20 μ g/ml propidium iodide in PBS cells, fixed in 70% ethanol) indicated that the DNA content of 60% of the nonadherent population was less than 2n. The cells appeared as a gaussian peak to the left of G₀G₁, which is characteristic of apoptosis (FIG. 8). Furthermore, the nonadherent cells appeared to be arrested in the G1 phase. These flow cytometric findings correlated with the inhibition of cell growth observed following antisense therapy. Furthermore, the C8161 cells showed no significant growth for three days (72 hours) following exposure to antisense oligonucleosides while MSN2 control treatments showed no alterations in transformed cell growth. The effects on C8161 cells specifically correlated with the antisense attenuation of p125^{FAK} expression (FIG. 4). These observations are similar to the anoikis phenomenon described by Frisch, et al. (J. Cell Biology 124:619-626 (1994)) and is a further indication that FAK may play a role in regulating these events.

Similar growth inhibition effects were observed in RD and BT20 cells treated with FAK antisense oligonucleosides. For example, the DNA histogram for nonadherent RD cells, when compared to the adherent group, contained a larger population with low DNA content, indicative of apoptosis (FIG. 9A). Indeed, 60% of these cells had a DNA content of less than 2n, as measured by FACScan analysis. In contrast, the majority of adherent cells treated with antisense oligonucleosides contained a normal DNA content (FIG. 9B).

Because flow cytometric analysis suggested that these antisense treated cells were apoptotic, several other apoptotic criteria were assessed on the nonadherent cells. One of the hallmarks of apoptotic cell death is endonuclease cleavage of genomic DNA into nucleosomal size fragments of 200 bp.

Following DNA extraction from both the adherent and nonadherent antisense treated cells and from cells treated with missense oligonucleoside, the non-adherent population demonstrated nucleosomal fragmentation. In contrast, the adherent cells which did not have attenuated FAK expression maintained intact DNA (FIG. 9C).

A further distinguishing event in apoptosis is the degradation of the nucleus into vesicles. When several of the nonadherent cells with different morphologies were examined, a few cells appeared to be early in the apoptotic process, with their nuclear envelope still evident but with condensation and margination of the chromatin (FIG. 10A). However, the majority of the cells in the nonadherent population had the morphology demonstrated in FIG. 10B, in which several apoptotic bodies were evident, and the nuclear envelope had dispersed while the plasma membrane remained intact. Giemsa staining of the cells allowed quantitation of the relative number of cells undergoing apoptosis in AS2 treated cells (FIG. 10C).

Following staining of both the adherent and nonadherent cell populations, the percentage of apoptotic cells was determined by counting the number of apoptotic cells versus the total number of cells sighted in ten microscopic fields (400 \times magnification). Cells treated with MSN2 showed a negligible percentage of apoptotic cells, while cells treated with AS2 had a significantly higher percentage of apoptosis in each of the cell lines studied (see FIG. 11 and Table 3). These results indicate that attenuation of p125^{FAK} causes cells to undergo apoptosis.

The percentage of apoptotic cells in the RD, C8161 and BT474 cell lines following treatment with AS2 and MSN2 controls are summarized in Table 3 below. No measurable apoptosis was observed in any of these cell lines using the 5bp missense control.

TABLE 3. PERCENT OF APOPTOSIS

Cell Type	Oligonucleoside Type	
	AS2	MSN2
RD	45.5%	0.0%
C8161	34.1%	0.0%
BT474	30.7%	0.0%

F. Inhibition of Tumor Cell Motility by FAK Antisense Compounds

Tumor cells interact with basement membranes in a manner fundamentally different from normal cells. The results presented above suggested that antisense attenuation of p125^{FAK} expression might interrupt the ability of tumor cells to bind to their adjacent basement membrane, an initial requirement in the sequence of events leading to invasion. A subsequent step in the invasion process involves alterations in cellular motility which allow cells to actually propel themselves across the basement membrane and enter the interstitial stroma. To assess the role of FAK in these events, we used an *in vitro* cell invasion assay and determined the changes in the migration patterns of C8161 cells, which are known to be highly invasive, after attenuation of p125^{FAK} expression.

The invasion assay allowed measurement of the invasive potential of cells through a reconstituted basement membrane in a modified Boyden chamber. Biocoat Matrigel Invasion Chambers (Becton Dickinson) were rehydrated over 2 hours by adding 2 ml of warm Opti-Mem and placed into individual wells of Falcon six-well culture plates. Conditioned medium was obtained by incubating human fibroblasts for 24 hours in Opti-Mem. This medium was used as a source of chemoattractants and was placed in the lower compartment of the Boyden chambers. 2 x 10⁵ C8161 cells pre-treated with FAK antisense or 5bp missense oligonucleosides were suspended in Opti-Mem containing 10% FCS and added

to the rehydrated upper chambers. Assays were carried out at 37°C in 5% CO₂. At the end of the incubation (about 24 hours), the cells on the upper surface of the filter were completely removed by wiping with a cotton swab under direct microscopic visualization. The filters were fixed in methanol and stained with hematoxylin and eosin. Cells from various areas of the lower surface were counted to correlate cell invasion of the reconstituted basement membrane. Each assay was performed in triplicate.

As shown in FIG. 12, the antisense treated cells demonstrated a dramatically lower invasive potential ($5.8 \pm 4.3\%$) compared to the missense treated control ($40.6 \pm 5.2\%$). An emerging theme in the study of tumor invasion is that, in addition to unrestrained growth, tumor cells display an imbalanced regulation of motility and proteolysis. These *in vitro* results suggest that FAK may be closely involved in the former of these two critical processes.

G. Inhibition of Anchorage-Independent Growth Properties by FAK Antisense Compounds

In addition to motility changes, invasive and metastatic cells develop enhanced anchorage-independent growth properties. This phenomenon is most apparent in human malignancy during the events leading to tumor dissemination in processes such as carcinomatosis, as well as in tumor cell colony formation in metastatic target organs. We assessed the anchorage-independent growth properties in FAK antisense treated cells by measuring their ability to form colonies in soft agar.

Two different tumor cell lines (C8161 and RD) were used in these studies. Oligonucleoside treated cells were seeded at a density of 5×10^4 cells per plate in a 0.33% top agarose layer in RPMI-1640 supplemented with 10% fetal bovine serum. The semisolid cell containing agar was layered onto 0.5% hard agar and incubated on scored tissue culture dishes (60 mm in diameter) in a humidified,

5% CO₂ atmosphere at 37°C. The dishes were fed once every several days with 1.0 ml of 1X medium. Colony formation efficiency was determined after two weeks in triplicate, blinded fashion by phase contrast microscopy counting all colonies larger than 70mm in diameter.

5 The results of these studies are shown in Table 4, wherein the values represent the number of colonies in soft agar along with a calculated colony forming efficiency (total colonies per plate/total cells per plate, times 100). Following specific attenuation of FAK expression using either the FAK1AS or FAK2AS antisense compounds, a marked reduction in colony formation in soft
10 agar was seen compared to cells treated with the control missense oligonucleoside MSN2. After two weeks of culture there was a 81% reduction in colony formation efficiency in the C8161 cells treated with a FAK antisense oligonucleosides and a 85% reduction in colony formation efficiency in the RD
15 cells treated with FAK antisense compared to cells treated with missense oligonucleosides. These results confirm that the loss of adhesion seen in cells after FAK antisense treatment is associated with reduced anchorage-independent growth.

TABLE 4. ANCHORAGE-INDEPENDENT GROWTH OF FAK
ANTISENSE TREATED CELLS

	<u>C8161</u>	<u>Total # Colonies</u>	<u>Colony Forming Efficiency</u>
5	MSN2	29,818 (± 907)	59.6
	FAK2AS	5,759 (± 571)	11.5
	<u>RD</u>	<u>Total # Colonies</u>	<u>Colony Forming Efficiency</u>
	MSN2	10,205 (± 2586)	20.4
	FAK2AS	1544 (± 630)	3.1

10

H. Inhibition of Tumor Formation in Nude Mice by FAK Antisense Compounds

15 The ability of FAK antisense compounds to inhibit tumor formation in
 athymic nude mice was also tested. Four-week old female athymic nude mice
 (Harlan Sprague-Dawley) were used in this study. Animals were maintained
 under the guidelines of the National Institutes of Health and The University of
 North Carolina School of Medicine. Mice were injected s.c. in the dorsolateral
 left flank with cells (RD or C8161, 2×10^6) suspended in HBSS. Prior to
 20 injection the cells were either treated with FAK antisense (FAK1AS or
 FAK2AS) or missense control (MSN2) as previously described. Tumor growth
 was monitored serially beginning several days after injection. Two perpendicular
 measurements of the diameter of any palpable nodule were obtained, and an
 estimated volume was calculated as $lw^2/2$. The animal was sacrificed at the end
 25 of the experiment and examined for any intrusion of tumor through the body wall
 or evidence of metastases to various body organs. The tumors were removed and
 protein extracted for FAK analysis.

As shown in FIG. 13, there was a significant lag time (almost 2 weeks) in the development of tumors using both RD and C8161 treated cells. This was surprising since related studies (see FIG. 4) had shown that such cells regain their FAK expression capability after seventy-two hours.

5 Inhibition of tumor growth was also tested *in vivo* by continuous delivery of antisense or missense oligonucleosides. Twelve nude mice were injected s.c. in the dorsolateral left flank with 2×10^6 C8161 cells and allowed to grow for 7 days until an approximately 1 cm lesion formed. Alzet 2002 osmotic pumps were surgically implanted s.c. over the right scapula of the mice and loaded to
10 provide 12.5 mg/kg/day of the oligonucleoside over a 14-day treatment period. Six mice were treated with antisense oligonucleoside; the other six mice were treated with a missense control oligonucleoside. Two of the mice died within the first 3 days and another died at 11 days, but evaluation of tumor volume at 14 days showed significant tumor inhibition in the antisense-treated animals,
15 compared to those treated with the missense control. The results of the *in vivo* studies are summarized in Table 5 below, for the surviving mice (5 surviving mice for the antisense oligonucleoside; 4 surviving mice for the missense oligonucleoside). No gross liver or pulmonary metastasis was observed. This was further confirmed in the case of pulmonary tissue by blinded, independent
20 histopathological examination of fixed lung tissue sections.

TABLE 5. INHIBITION OF TUMOR GROWTH IN NUDE MICE USING FAK ANTISENSE COMPOUNDS

	Antisense Tumor Volume (mm ³)	Missense Tumor Volume (mm ³)
5	125	750
	125	350
	100	500
	63	650
10	125	
	Mean 108 ± 27	Mean 563 ± 175

The foregoing results show that increased levels of p125^{FAK} are associated with tumor invasion and metastasis and that disruption of this pathway by attenuating p125^{FAK} expression with FAK antisense oligonucleosides significantly inhibits cellular adhesion, motility and anchorage independence. This data also points to FAK as a mediator of the processes which are downstream from other signaling molecules such as c-src and fyn.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that various changes and modifications may be made thereto, and various equivalents used, without departing from the spirit or scope of the claims. Therefore, the foregoing description should not be construed to limit the scope of the present invention, which is set forth in the appended claims.

40

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: CANCE, William G.

LIU, Edison T.

OWENS, Lewis V.

(ii) TITLE OF INVENTION: Oligonucleoside Compounds and Methods
for Inhibiting Tumor Growth, Invasion
and Metastasis

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Lyon & Lyon

(B) STREET: 633 West Fifth Street, Suite 4700

(C) CITY: Los Angeles

(D) STATE: CA

(E) COUNTRY: U.S.A.

(F) ZIP: 90071-2066

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/276,843

(B) FILING DATE: 18-JULY-1994

(C) CLASSIFICATION: 03A1/0912

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Meier, Paul H.

(B) REGISTRATION NUMBER: 32,274

(C) REFERENCE/DOCKET NUMBER: 208/061

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 213/489-1600

(B) TELEFAX: 213/955-0440

(C) TELEX: 67-3510

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1052 amino acids

(B) TYPE: amino acid

41

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(ix) FEATURE:

(A) NAME/KEY: HUMPAK (human FAK)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

Met Ala Ala Ala Tyr Leu Asp Pro Asn Leu Asn His Thr Pro Asn Ser
 1           5           10           15
Ser Thr Lys Thr His Leu Gly Thr Gly Met Glu Arg Ser Pro Gly Ala
          20           25           30
Met Glu Arg Val Leu Lys Val Phe His His Phe Glu Ser Ser Ser Glu
          35           40           45
Pro Thr Thr Trp Ala Ser Ile Ile Arg His Gly Asp Ala Thr Asp Val
          50           55           60
Arg Gly Ile Ile Gln Lys Ile Val Asp Ser His Lys Val Lys His Val
          65           70           75           80
Ala Cys Tyr Gly Phe Arg Leu Ser His Leu Arg Ser Glu Glu Val His
          85           90           95
Trp Leu His Val Asp Met Gly Val Ser Ser Val Arg Glu Lys Tyr Glu
          100          105          110
Leu Ala His Pro Pro Glu Glu Trp Lys Tyr Glu Leu Arg Ile Arg Tyr
          115          120          125
Leu Pro Lys Gly Phe Leu Asn Gln Phe Thr Glu Asp Lys Pro Thr Leu
          130          135          140
Asn Phe Phe Tyr Gln Gln Val Lys Ser Asp Tyr Met Gln Glu Ile Ala
          145          150          155          160
Asp Gln Val Asp Gln Glu Ile Ala Leu Lys Leu Gly Cys Leu Glu Ile
          165          170          175
Arg Arg Ser Tyr Trp Glu Met Arg Gly Asn Ala Leu Glu Lys Lys Ser
          180          185          190
Asn Tyr Glu Val Leu Glu Lys Asp Val Gly Leu Lys Arg Phe Phe Pro
          195          200          205
Lys Ser Leu Leu Asp Ser Val Lys Ala Lys Thr Leu Arg Lys Leu Ile
          210          215          220
Gln Gln Thr Phe Arg Gln Phe Ala Asn Leu Asn Arg Glu Glu Ser Ile
          225          230          235          240

```

Leu	Lys	Phe	Phe	Glu	Ile	Leu	Ser	Pro	Val	Tyr	Arg	Phe	Asp	Lys	Glu
				245						250				255	
Cys	Phe	Lys	Cys	Ala	Leu	Gly	Ser	Ser	Trp	Ile	Ile	Ser	Val	Glu	Leu
				260					265					270	
Ala	Ile	Gly	Pro	Glu	Glu	Gly	Ile	Ser	Tyr	Leu	Thr	Asp	Lys	Gly	Cys
				275				280					285		
Asn	Pro	Thr	His	Leu	Ala	Asp	Phe	Thr	Gln	Val	Gln	Thr	Ile	Gln	Tyr
				290				295				300			
Ser	Asn	Ser	Glu	Asp	Lys	Asp	Arg	Lys	Gly	Met	Leu	Gln	Leu	Lys	Ile
305					310					315					320
Ala	Gly	Ala	Pro	Glu	Pro	Leu	Thr	Val	Thr	Ala	Pro	Ser	Leu	Thr	Ile
				325					330					335	
Ala	Glu	Asn	Met	Ala	Asp	Leu	Ile	Asp	Gly	Tyr	Cys	Arg	Leu	Val	Asn
				340				345					350		
Gly	Thr	Ser	Gln	Ser	Phe	Ile	Ile	Arg	Pro	Gln	Lys	Glu	Gly	Glu	Arg
				355				360					365		
Ala	Leu	Pro	Ser	Ile	Pro	Lys	Leu	Ala	Asn	Ser	Glu	Lys	Gln	Gly	Met
				370			375					380			
Arg	Thr	His	Ala	Val	Ser	Val	Ser	Glu	Thr	Asp	Asp	Tyr	Ala	Glu	Ile
385					390					395					400
Ile	Asp	Glu	Glu	Asp	Thr	Tyr	Thr	Met	Pro	Ser	Thr	Arg	Asp	Tyr	Glu
				405					410					415	
Ile	Gln	Arg	Glu	Arg	Ile	Glu	Leu	Gly	Arg	Cys	Ile	Gly	Glu	Gly	Gln
				420				425					430		
Phe	Gly	Asp	Val	His	Gln	Gly	Ile	Tyr	Met	Ser	Pro	Glu	Asn	Pro	Ala
				435				440					445		
Leu	Ala	Val	Ala	Ile	Lys	Thr	Cys	Lys	Asn	Cys	Thr	Ser	Asp	Ser	Val
				450			455					460			
Arg	Glu	Lys	Phe	Leu	Gln	Glu	Ala	Leu	Thr	Met	Arg	Gln	Phe	Asp	His
465					470					475					480
Pro	His	Ile	Val	Lys	Leu	Ile	Gly	Val	Ile	Thr	Glu	Asn	Pro	Val	Trp
				485					490					495	
Ile	Ile	Met	Glu	Leu	Cys	Thr	Leu	Gly	Glu	Leu	Arg	Ser	Phe	Leu	Gln
				500					505					510	
Val	Arg	Lys	Tyr	Ser	Leu	Asp	Leu	Ala	Ser	Leu	Ile	Leu	Tyr	Ala	Tyr
				515				520						525	

43

Gln Leu Ser Thr Ala Leu Ala Tyr Leu Glu Ser Lys Arg Phe Val His
 530 535 540
 Arg Asp Ile Ala Ala Arg Asn Val Leu Val Ser Ser Asn Asp Cys Val
 545 550 555 560
 Lys Leu Gly Asp Phe Gly Leu Ser Arg Tyr Met Glu Asp Ser Thr Tyr
 565 570 575
 Tyr Lys Ala Ser Lys Gly Lys Leu Pro Ile Lys Trp Met Ala Pro Glu
 580 585 590
 Ser Ile Asn Phe Arg Arg Phe Thr Ser Ala Ser Asp Val Trp Met Phe
 595 600 605
 Gly Val Cys Met Trp Glu Ile Leu Met His Gly Val Lys Pro Phe Gln
 610 615 620
 Gly Val Lys Asn Asn Asp Val Ile Gly Arg Ile Glu Asn Gly Glu Arg
 625 630 635 640
 Leu Pro Met Pro Pro Asn Cys Pro Pro Thr Leu Tyr Ser Leu Met Thr
 645 650 655
 Lys Cys Trp Ala Tyr Asp Pro Ser Arg Arg Pro Arg Phe Thr Glu Leu
 660 665 670
 Lys Ala Gln Leu Ser Thr Ile Leu Glu Glu Glu Lys Ala Gln Gln Glu
 675 680 685
 Glu Arg Met Arg Met Glu Ser Arg Arg Gln Ala Thr Val Ser Trp Asp
 690 695 700
 Ser Gly Gly Ser Asp Glu Ala Pro Pro Lys Pro Ser Arg Pro Gly Tyr
 705 710 715 720
 Pro Ser Pro Arg Ser Ser Glu Gly Phe Tyr Pro Ser Pro Gln His Met
 725 730 735
 Val Gln Thr Asn His Tyr Gln Val Ser Gly Tyr Pro Gly Ser His Gly
 740 745 750
 Ile Thr Ala Met Ala Gly Ser Ile Tyr Pro Gly Gln Ala Ser Leu Leu
 755 760 765
 Asp Gln Thr Asp Ser Trp Asn His Arg Pro Gln Glu Ile Ala Met Trp
 770 775 780
 Gln Pro Asn Val Glu Asp Ser Thr Val Leu Asp Leu Arg Gly Ile Gly
 785 790 795 800
 Gln Val Leu Pro Thr His Leu Met Glu Glu Arg Leu Ile Arg Gln Gln
 805 810 815

44

Gln Glu Met Glu Glu Asp Gln Arg Trp Leu Glu Lys Glu Glu Arg Phe
 820 825 830
 Leu Lys Pro Asp Val Arg Leu Ser Arg Gly Ser Ile Asp Arg Glu Asp
 835 840 845
 Gly Ser Leu Gln Gly Pro Ile Gly Asn Gln His Ile Tyr Gln Pro Val
 850 855 860
 Gly Lys Pro Asp Pro Ala Ala Pro Pro Lys Lys Pro Pro Arg Pro Gly
 865 870 875 880
 Ala Pro Gly His Leu Gly Ser Leu Ala Ser Leu Ser Ser Pro Ala Asp
 885 890 895
 Ser Tyr Asn Glu Gly Val Lys Leu Gln Pro Gln Glu Ile Ser Pro Pro
 900 905 910
 Pro Thr Ala Asn Leu Asp Arg Ser Asn Asp Lys Val Tyr Glu Asn Val
 915 920 925
 Thr Gly Leu Val Lys Ala Val Ile Glu Met Ser Ser Lys Ile Gln Pro
 930 935 940
 Ala Pro Pro Glu Glu Tyr Val Pro Met Val Lys Glu Val Gly Leu Ala
 945 950 955 960
 Leu Arg Thr Leu Leu Ala Thr Val Asp Glu Thr Ile Pro Leu Leu Pro
 965 970 975
 Ala Ser Thr His Arg Glu Ile Glu Met Ala Gln Lys Leu Leu Asn Ser
 980 985 990
 Asp Leu Gly Glu Leu Ile Asn Lys Met Lys Leu Ala Gln Gln Tyr Val
 995 1000 1005
 Met Thr Ser Leu Gln Gln Glu Tyr Lys Lys Gln Met Leu Thr Ala Ala
 1010 1015 1020
 His Ala Leu Ala Val Asp Ala Lys Asn Leu Leu Asp Val Ile Asp Gln
 1025 1030 1035 1040
 Ala Arg Leu Lys Met Leu Gly Gln Thr Arg Pro His
 1045 1050

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1052 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: No

45

(iv) ANTI-SENSE: No

(ix) FEATURE:

(A) NAME/KEY: MUSFAK (mouse FAK)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Ala Ala Ala Tyr Leu Asp Pro Asn Leu Asn His Thr Pro Ser Ser
 1           5           10           15
Ser Thr Lys Thr His Leu Gly Thr Gly Met Glu Arg Ser Pro Gly Ala
          20           25           30
Met Glu Arg Val Leu Lys Val Phe His His Phe Glu Ser Ser Ser Glu
          35           40           45
Pro Thr Thr Trp Ala Ser Ile Ile Arg His Gly Asp Ala Thr Asp Val
          50           55           60
Arg Gly Ile Ile Gln Lys Ile Val Asp Ser His Lys Val Lys His Val
        65           70           75           80
Ala Cys Tyr Gly Phe Arg Leu Ser His Leu Arg Ser Glu Glu Val His
          85           90           95
Trp Leu His Val Asp Met Gly Val Ser Ser Val Arg Glu Lys Tyr Glu
          100          105          110
Leu Ala His Pro Pro Glu Glu Trp Lys Tyr Glu Leu Arg Ile Arg Tyr
          115          120          125
Leu Pro Lys Gly Phe Leu Asn Gln Phe Thr Glu Asp Lys Pro Thr Leu
          130          135          140
Asn Phe Phe Tyr Gln Gln Val Lys Ser Asp Tyr Met Gln Glu Ile Ala
          145          150          155          160
Asp Gln Val Asp Gln Glu Ile Ala Leu Lys Leu Gly Cys Leu Glu Ile
          165          170          175
Arg Arg Ser Tyr Trp Glu Met Arg Gly Asn Ala Leu Glu Lys Lys Ser
          180          185          190
Asn Tyr Glu Val Leu Glu Lys Asp Val Gly Leu Lys Arg Phe Phe Pro
          195          200          205
Lys Ser Leu Leu Asp Ser Val Lys Ala Lys Thr Leu Arg Lys Leu Ile
          210          215          220
Gln Gln Thr Phe Arg Gln Phe Ala Asn Leu Asn Arg Glu Glu Ser Ile
          225          230          235          240
Leu Lys Phe Phe Glu Ile Leu Ser Pro Val Tyr Arg Phe Asp Lys Glu
          245          250          255

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46

Cys Phe Lys Cys Ala Leu Gly Ser Ser Trp Ile Ile Ser Val Glu Leu
 260 265 270
 Ala Ile Gly Pro Glu Glu Gly Ile Ser Tyr Leu Thr Asp Lys Gly Cys
 275 280 285
 Asn Pro Thr His Leu Ala Asp Phe Asn Gln Val Gln Thr Ile Gln Tyr
 290 295 300
 Ser Asn Ser Glu Asp Lys Asp Arg Lys Gly Met Leu Gln Leu Lys Ile
 305 310 315 320
 Ala Gly Ala Pro Glu Pro Leu Thr Val Thr Ala Pro Ser Leu Thr Ile
 325 330 335
 Ala Glu Asn Met Ala Asp Leu Ile Asp Gly Tyr Cys Arg Leu Val Asn
 340 345 350
 Gly Ala Thr Gln Ser Phe Ile Ile Arg Pro Gln Lys Glu Gly Glu Arg
 355 360 365
 Ala Leu Pro Ser Ile Pro Lys Leu Ala Asn Ser Glu Lys Gln Gly Met
 370 375 380
 Arg Thr His Ala Val Ser Val Ser Glu Thr Asp Asp Tyr Ala Glu Ile
 385 390 395 400
 Ile Asp Glu Glu Asp Thr Tyr Thr Met Pro Ser Thr Arg Asp Tyr Glu
 405 410 415
 Ile Gln Arg Glu Arg Ile Glu Leu Gly Arg Cys Ile Gly Glu Gly Gln
 420 425 430
 Phe Gly Asp Val His Gln Gly Val Tyr Leu Ser Pro Glu Asn Pro Ala
 435 440 445
 Leu Ala Val Ala Ile Lys Thr Cys Lys Asn Cys Thr Ser Asp Ser Val
 450 455 460
 Arg Glu Lys Phe Leu Gln Glu Ala Leu Thr Met Arg Gln Phe Asp His
 465 470 475 480
 Pro His Ile Val Lys Leu Ile Gly Val Ile Thr Glu Asn Pro Val Trp
 485 490 495
 Ile Ile Met Glu Leu Cys Thr Leu Gly Glu Leu Arg Ser Phe Leu Gln
 500 505 510
 Val Arg Lys Tyr Ser Leu Asp Leu Ala Ser Leu Ile Leu Tyr Ala Tyr
 515 520 525
 Gln Leu Ser Thr Ala Leu Ala Tyr Leu Glu Ser Lys Arg Phe Val His
 530 535 540

47

Arg Asp Ile Ala Ala Arg Asn Val Leu Val Ser Ser Asn Asp Cys Val
 545 550 555 560
 Lys Leu Gly Asp Phe Gly Leu Ser Arg Tyr Met Glu Asp Ser Thr Tyr
 565 570 575
 Tyr Lys Ala Ser Lys Gly Lys Leu Pro Ile Lys Trp Met Ala Pro Glu
 580 585 590
 Ser Ile Asn Phe Arg Arg Phe Thr Ser Ala Ser Asp Val Trp Met Phe
 595 600 605
 Gly Val Cys Met Trp Glu Ile Leu Met His Gly Val Lys Pro Phe Gln
 610 615 620
 Gly Val Lys Asn Asn Asp Val Ile Gly Arg Ile Glu Asn Gly Glu Arg
 625 630 635 640
 Leu Pro Met Pro Pro Asn Cys Pro Pro Thr Leu Tyr Ser Leu Met Thr
 645 650 655
 Lys Cys Trp Ala Tyr Asp Pro Ser Arg Arg Pro Arg Phe Thr Glu Leu
 660 665 670
 Lys Ala Gln Leu Ser Thr Ile Leu Glu Glu Glu Lys Val Gln Gln Glu
 675 680 685
 Glu Arg Met Arg Met Glu Ser Arg Arg Gln Ala Thr Val Ser Trp Asp
 690 695 700
 Ser Gly Gly Ser Asp Glu Ala Pro Pro Lys Pro Ser Arg Pro Gly Tyr
 705 710 715 720
 Pro Ser Pro Arg Ser Ser Glu Gly Phe Tyr Pro Ser Pro Gln His Met
 725 730 735
 Val Gln Thr Asn His Tyr Gln Val Ser Gly Tyr Pro Gly Ser His Gly
 740 745 750
 Ile Pro Ala Met Ala Gly Ser Ile Tyr Gln Gly Gln Ala Ser Leu Leu
 755 760 765
 Asp Gln Thr Glu Leu Trp Asn His Arg Pro Gln Glu Met Ser Met Trp
 770 775 780
 Gln Pro Ser Val Glu Asp Ser Ala Ala Leu Asp Leu Arg Gly Met Gly
 785 790 795 800
 Gln Val Leu Pro Pro His Leu Met Glu Glu Arg Leu Ile Arg Gln Gln
 805 810 815
 Gln Glu Met Glu Glu Asp Gln Arg Trp Leu Glu Lys Glu Glu Arg Phe
 820 825 830

48

Leu Lys Pro Asp Val Arg Leu Ser Arg Gly Ser Ile Asp Arg Glu Asp
 835 840 845
 Gly Ser Phe Gln Gly Pro Thr Gly Asn Gln His Ile Tyr Gln Pro Val
 850 855 860
 Gly Lys Pro Asp Pro Ala Ala Pro Pro Lys Lys Pro Pro Arg Pro Gly
 865 870 875 880
 Ala Pro Gly His Leu Ser Asn Leu Ser Ser Ile Ser Ser Pro Ala Asp
 885 890 895
 Ser Tyr Asn Glu Gly Val Lys Leu Gln Pro Gln Glu Ile Ser Pro Pro
 900 905 910
 Pro Thr Ala Asn Leu Asp Arg Ser Asn Asp Lys Val Tyr Glu Asn Val
 915 920 925
 Thr Gly Leu Val Lys Ala Val Ile Glu Met Ser Ser Lys Ile Gln Pro
 930 935 940
 Ala Pro Pro Glu Glu Tyr Val Pro Met Val Lys Glu Val Gly Leu Ala
 945 950 955 960
 Leu Arg Thr Leu Leu Ala Thr Val Asp Glu Thr Ile Pro Ala Leu Pro
 965 970 975
 Ala Ser Thr His Arg Glu Ile Glu Met Ala Gln Lys Leu Leu Asn Ser
 980 985 990
 Asp Leu Gly Glu Leu Ile Ser Lys Met Lys Leu Ala Gln Gln Tyr Val
 995 1000 1005
 Met Thr Ser Leu Gln Gln Glu Tyr Lys Lys Gln Met Leu Thr Ala Ala
 1010 1015 1020
 His Ala Leu Ala Val Asp Ala Lys Asn Leu Leu Asp Val Ile Asp Gln
 1025 1030 1035 1040
 Ala Arg Leu Lys Met Leu Gly Gln Thr Arg Pro His
 1045 1050

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1053 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(ix) FEATURE:

49

(A) NAME/KEY: CHKFAK (chicken FAK)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Ala Ala Ala Tyr Leu Asp Pro Asn Leu Asn His Thr Pro Ser Ser
 1           5           10           15
Ser Ala Lys Thr His Leu Gly Thr Gly Met Glu Arg Ser Pro Gly Ala
          20           25           30
Met Glu Arg Val Leu Lys Val Phe His Tyr Phe Glu Asn Ser Ser Glu
          35           40           45
Pro Thr Thr Trp Ala Ser Ile Ile Arg His Gly Asp Ala Thr Asp Val
          50           55           60
Arg Gly Ile Ile Gln Lys Ile Val Asp Cys His Lys Val Lys Asn Val
65           70           75           80
Ala Cys Tyr Gly Leu Arg Leu Ser His Leu Gln Ser Glu Glu Val His
          85           90           95
Trp Leu His Leu Asp Met Gly Val Ser Asn Val Arg Glu Lys Phe Glu
          100          105          110
Leu Ala His Pro Pro Glu Glu Trp Lys Tyr Glu Leu Arg Ile Arg Tyr
          115          120          125
Leu Pro Lys Gly Phe Leu Asn Gln Phe Thr Glu Asp Lys Pro Thr Leu
          130          135          140
Asn Phe Phe Tyr Gln Gln Val Lys Asn Asp Tyr Met Leu Glu Ile Ala
145          150          155          160
Asp Gln Val Asp Gln Glu Ile Ala Leu Lys Leu Gly Cys Leu Glu Ile
          165          170          175
Arg Arg Ser Tyr Gly Glu Met Arg Gly Asn Ala Leu Glu Lys Lys Ser
          180          185          190
Asn Tyr Glu Val Leu Glu Lys Asp Val Gly Leu Arg Arg Phe Phe Pro
          195          200          205
Lys Ser Leu Leu Asp Ser Val Lys Ala Lys Thr Leu Arg Lys Leu Ile
          210          215          220
Gln Gln Thr Phe Arg Gln Phe Ala Asn Leu Asn Arg Glu Glu Ser Ile
225          230          235          240
Leu Lys Phe Phe Glu Ile Leu Ser Pro Val Tyr Arg Phe Asp Lys Glu
          245          250          255
Cys Phe Lys Cys Ala Leu Gly Ser Ser Trp Ile Ile Ser Val Glu Leu
          260          265          270

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50

Ala Ile Gly Pro Glu Glu Gly Ile Ser Tyr Leu Thr Asp Lys Gly Ala
 275 280 285
 Asn Pro Thr His Leu Ala Asp Phe Asn Gln Val Gln Thr Ile Gln Tyr
 290 295 300
 Ser Asn Ser Glu Asp Lys Asp Arg Lys Gly Met Leu Gln Leu Lys Ile
 305 310 315 320
 Ala Gly Ala Pro Glu Pro Leu Thr Val Thr Ala Pro Ser Leu Thr Ile
 325 330 335
 Ala Glu Asn Met Ala Asp Leu Ile Asp Gly Tyr Cys Arg Leu Val Asn
 340 345 350
 Gly Ala Thr Gln Ser Phe Ile Ile Arg Pro Gln Lys Glu Gly Glu Arg
 355 360 365
 Ala Leu Pro Ser Ile Pro Lys Leu Ala Asn Asn Glu Lys Gln Gly Val
 370 375 380
 Arg Ser His Thr Val Ser Val Ser Glu Thr Asp Asp Tyr Ala Glu Ile
 385 390 395 400
 Ile Asp Glu Glu Asp Thr Tyr Thr Met Pro Ser Thr Arg Asp Tyr Glu
 405 410 415
 Ile Gln Arg Glu Arg Ile Glu Leu Gly Arg Cys Ile Gly Glu Gly Gln
 420 425 430
 Phe Gly Asp Val His Gln Gly Ile Tyr Met Ser Pro Glu Asn Pro Ala
 435 440 445
 Met Ala Val Ala Ile Lys Thr Cys Lys Asn Cys Thr Ser Asp Ser Val
 450 455 460
 Arg Glu Lys Phe Leu Gln Glu Ala Leu Thr Met Arg Gln Phe Asp His
 465 470 475 480
 Pro His Ile Val Lys Leu Ile Gly Val Ile Thr Glu Asn Pro Val Trp
 485 490 495
 Ile Ile Met Glu Leu Cys Thr Leu Gly Glu Leu Arg Ser Phe Leu Gln
 500 505 510
 Val Arg Lys Phe Ser Leu Asp Leu Ala Ser Leu Ile Leu Tyr Ala Tyr
 515 520 525
 Gln Leu Ser Thr Ala Leu Ala Tyr Leu Glu Ser Lys Arg Phe Val His
 530 535 540
 Arg Asp Ile Ala Ala Arg Asn Val Leu Val Ser Ala Thr Asp Cys Val
 545 550 555 560

51

Lys Leu Gly Asp Phe Gly Leu Ser Arg Tyr Met Glu Asp Ser Thr Tyr
 565 570 575
 Tyr Lys Ala Ser Lys Gly Lys Leu Pro Ile Lys Trp Met Ala Pro Glu
 580 585 590
 Ser Ile Asn Phe Arg Arg Phe Thr Ser Ala Ser Asp Val Trp Met Phe
 595 600 605
 Gly Val Cys Met Trp Glu Ile Leu Met His Gly Val Lys Pro Phe Gln
 610 615 620
 Gly Val Lys Asn Asn Asp Val Ile Gly Arg Ile Glu Asn Gly Glu Arg
 625 630 635 640
 Leu Pro Met Pro Pro Asn Cys Pro Pro Thr Leu Tyr Ser Leu Met Thr
 645 650 655
 Lys Cys Trp Ala Tyr Asp Pro Ser Arg Arg Pro Arg Phe Thr Glu Leu
 660 665 670
 Lys Ala Gln Leu Ser Thr Ile Leu Glu Glu Glu Lys Leu Gln Gln Glu
 675 680 685
 Glu Arg Met Arg Met Glu Ser Arg Arg Gln Val Thr Val Ser Trp Asp
 690 695 700
 Ser Gly Gly Ser Asp Glu Ala Pro Pro Lys Pro Ser Arg Pro Gly Tyr
 705 710 715 720
 Pro Ser Pro Arg Ser Ser Glu Gly Phe Tyr Pro Ser Pro Gln His Met
 725 730 735
 Val Gln Pro Asn His Tyr Gln Val Ser Gly Tyr Ser Gly Ser His Gly
 740 745 750
 Ile Pro Ala Met Ala Gly Ser Ile Tyr Pro Gly Gln Ala Ser Leu Leu
 755 760 765
 Asp Gln Thr Asp Ser Trp Asn His Arg Pro Gln Glu Val Ser Ala Trp
 770 775 780
 Gln Pro Asn Met Glu Asp Ser Gly Thr Leu Asp Val Arg Gly Met Gly
 785 790 795 800
 Gln Val Leu Pro Thr His Leu Met Glu Glu Arg Leu Ile Arg Gln Gln
 805 810 815
 Gln Glu Met Glu Glu Asp Gln Arg Trp Leu Glu Lys Glu Glu Arg Phe
 820 825 830
 Leu Val Met Lys Pro Asp Val Arg Leu Ser Arg Gly Ser Ile Glu Arg
 835 840 845

52

Glu Asp Gly Gly Leu Gln Gly Pro Ala Gly Asn Gln His Ile Tyr Gln
 850 855 860
 Pro Val Gly Lys Pro Asp His Ala Ala Pro Pro Lys Lys Pro Pro Arg
 865 870 875 880
 Pro Gly Ala Pro His Leu Gly Ser Leu Ala Ser Leu Asn Ser Pro Val
 885 890 895
 Asp Ser Tyr Asn Glu Gly Val Lys Ile Lys Pro Gln Glu Ile Ser Pro
 900 905 910
 Pro Pro Thr Ala Asn Leu Asp Arg Ser Asn Asp Lys Val Tyr Glu Asn
 915 920 925
 Val Thr Gly Leu Val Lys Ala Val Ile Glu Met Ser Ser Lys Ile Gln
 930 935 940
 Pro Ala Pro Pro Glu Glu Tyr Val Pro Met Val Lys Glu Val Gly Leu
 945 950 955 960
 Ala Leu Arg Thr Leu Leu Ala Thr Val Asp Glu Ser Leu Pro Val Leu
 965 970 975
 Pro Ala Ser Thr His Arg Glu Ile Glu Met Ala Gln Lys Leu Leu Asn
 980 985 990
 Ser Asp Leu Ala Glu Leu Ile Asn Lys Met Lys Leu Ala Gln Gln Tyr
 995 1000 1005
 Val Met Thr Ser Leu Gln Gln Glu Tyr Lys Lys Gln Met Leu Thr Ala
 1010 1015 1020
 Ala His Ala Leu Ala Val Asp Ala Lys Asn Leu Leu Asp Val Ile Asp
 1025 1030 1035 1040
 Gln Ala Arg Leu Lys Met Ile Ser Gln Ser Arg Pro His
 1045 1050

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(A) NAME/KEY: FAKIAS

(D) OTHER INFORMATION: inhibition of FAK expression

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ACACTTGAAG CATTCTTAT CAAA

24

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(A) NAME/KEY: FAK2AS

(D) OTHER INFORMATION: inhibition of FAK expression

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATAATCCAGC TTGAACCAAG

20

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(A) NAME/KEY: MSN1

(D) OTHER INFORMATION: 2-base mismatch control

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATAATCGAGC TTCAACCAAG

20

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: other nucleic acid
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: Yes
- (ix) FEATURE:
 - (A) NAME/KEY: MSN2
 - (D) OTHER INFORMATION: 5-base mismatch control
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATAATCGACG TTCAAGCAAG

20

- (9) INFORMATION FOR SEQ ID NO:8:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (iii) HYPOTHETICAL: No
 - (iv) ANTI-SENSE: Yes
 - (ix) FEATURE:
 - (A) NAME/KEY: WNT
 - (D) OTHER INFORMATION: nonsense control sequence
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGCCCGAGCA GGTGGGGCTC

20

- (10) INFORMATION FOR SEQ ID NO:9:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (iii) HYPOTHETICAL: No
 - (iv) ANTI-SENSE: Yes
 - (ix) FEATURE:
 - (A) NAME/KEY: (G)4
 - (D) OTHER INFORMATION: control sequence
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TATGCTCTGC CGGGGTCTTC GGGC

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What is claimed is:

1. An oligonucleoside compound for inhibiting expression of a focal adhesion kinase protein comprising from about 6 to about 40 linked nucleosides in a sequence that is complementary to a target region of a focal adhesion kinase mRNA.

2. The oligonucleoside compound of claim 1 wherein said focal adhesion kinase mRNA is a human mRNA.

3. The oligonucleoside compound of claim 2 wherein said human focal adhesion kinase mRNA is an mRNA overexpressed in a transformed human cell.

4. The oligonucleoside compound of claim 1 comprising from about 12 to about 30 linked nucleosides.

5. The oligonucleoside compound of claim 1 wherein at least one of the internucleoside linkage structures in the compound is a non-phosphodiester linkage that is resistant to degradation by an endogenous cellular nuclease.

1 6. The oligonucleoside compound of claim 5 wherein said at least one
2 non-phosphodiester linkage is a linkage selected from the group consisting of
3 phosphorothioate, phosphorodithioate, alkyl- or arylphosphonate, phosphor-
4 amidate, phosphotriester, alkyl- or arylphosphonothioate, aminoalkyl-
5 phosphonate, aminoalkylphosphonothioate, phosphorofluoridate, borano-
6 phosphate, silyl, formacetal, thioformacetal, morpholino and peptide-based
linkages.

7. The oligonucleoside compound of claim 5 comprising at least one phosphorothioate linkage.

8. The oligonucleoside compound of claim 7 comprising a plurality of phosphorothioate linkages.

9. The oligonucleoside compound of claim 1 wherein said focal adhesion kinase mRNA target region is in a 5'-untranslated portion of the mRNA.

10. The oligonucleoside compound of claim 1 wherein said focal adhesion kinase mRNA target region is in a coding portion of the mRNA.

11. The oligonucleoside compound of claim 2 wherein said complementary oligonucleoside sequence is specific for the target region of said human focal adhesion kinase mRNA, such that hybridization of the compound with unintended human nucleic acid sequences is minimized upon application of the compound to human cells.

12. A formulation comprising the oligonucleoside compound of any of claims 1, 2, 3 or 5 and a vehicle adapted to allow delivery of the compound to animal cells.

13. The formulation of claim 12 wherein said vehicle is a pharmaceutically acceptable carrier.

14. The formulation of claim 12 wherein said vehicle includes a material suitable to facilitate delivery of the oligonucleoside compound across a cell membrane of the animal subject.

15. The formulation of claim 14 wherein said material is a non-toxic lipid material.

16. A method of inhibiting growth of a transformed animal cell comprising administering to said animal cell the oligonucleoside compound of any of claims 1, 2, 3 or 5.

17. A method of inhibiting invasiveness of a transformed animal cell comprising administering to said animal cell the oligonucleoside compound of any of claims 1, 2, 3 or 5.

18. A method of inhibiting cell colony formation in transformed animal cells comprising administering to said animal cells the oligonucleoside compound of any of claims 1, 2, 3 or 5.

19. A method of inducing apoptosis of a transformed animal cell comprising administering to said animal cell the oligonucleoside compound of any of claims 1, 2, 3 or 5.

20. A method of reducing the rate of tumor formation attributable to transformed cells in an animal comprising administering to said animal the oligonucleoside compound of any of claims 1, 2, 3 or 5.

21. The method of claim 16 wherein said administration is to an animal subject in which transformed cells reside.

22. The method of claim 17 wherein said administration is to an animal subject in which transformed cells reside.

23. The method of claim 18 wherein said administration is to an animal subject in which transformed cells reside.

24. The method of claim 19 wherein said administration is to an animal subject in which transformed cells reside.

25. A method of treating cancer in a human comprising administering the oligonucleoside compound of any of claims 3, 4 or 5.

26. A method of treating cancer in a human comprising administering the formulation of claim 12.

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HUMFAK	1	MAAAYLDPNLTNPSS	TKTHLGTG	MERSPGAMERVL	KVFHFFESSES	PTTWASII	IRHG	DATDVRGI	IQKIVDSHKVKHV
MUSFAK	1	MAAAYLDPNLTNPSS	TKTHLGTG	MERSPGAMERVL	KVFHFFESSES	PTTWASII	IRHG	DATDVRGI	IQKIVDSHKVKHV
CHKFAK	1	MAAAYLDPNLTNPSS	AKTHLGTG	MERSPGAMERVL	KVFHYFENSSES	PTTWASII	IRHG	DATDVRGI	IQKIVDCHKVKNV
HUMFAK	81	ACYGFRLSHLRSEEV	HWLHVD	MGVSSVRE	KYELAH	PPPEWKYEL	IRYL	PKGFLNQ	FTEDEKPTL
MUSFAK	81	ACYGFRLSHLRSEEV	HWLHVD	MGVSSVRE	KYELAH	PPPEWKYEL	IRYL	PKGFLNQ	FTEDEKPTL
CHKFAK	81	ACYGLRSLHLQSEEV	HWLHLD	MGVSNVRE	KFELAH	PPPEWKYEL	IRYL	PKGFLNQ	FTEDEKPTL
HUMFAK	161	DQVDQEI	ALKLGC	LEIRRSY	WEMRGN	ALEKKSNYE	VLEKDV	GLKRFF	PKSLDS
MUSFAK	161	DQVDQEI	ALKLGC	LEIRRSY	WEMRGN	ALEKKSNYE	VLEKDV	GLKRFF	PKSLDS
CHKFAK	161	DQVDQEI	ALKLGC	LEIRRSY	GEMRGN	ALEKKSNYE	VLEKDV	GLKRFF	PKSLDS
HUMFAK	241	LKFFEIL	SPVYRFD	KECFK	CALGSSWII	ISVELA	IGPEEG	ISYLT	DKGNCN
MUSFAK	241	LKFFEIL	SPVYRFD	KECFK	CALGSSWII	ISVELA	IGPEEG	ISYLT	DKGNCN
CHKFAK	241	LKFFEIL	SPVYRFD	KECFK	CALGSSWII	ISVELA	IGPEEG	ISYLT	DKGNCN
HUMFAK	321	AGAPEL	TVTAP	SLTIA	ENMAD	LIDGYC	RVLN	GTQSFI	IRPQ
MUSFAK	321	AGAPEL	TVTAP	SLTIA	ENMAD	LIDGYC	RVLN	GTQSFI	IRPQ
CHKFAK	321	AGAPEL	TVTAP	SLTIA	ENMAD	LIDGYC	RVLN	GTQSFI	IRPQ
HUMFAK	401	IDEDTY	TMPSTR	DY	EIQ	RIE	LGRC	IGEG	QFGDV
MUSFAK	401	IDEDTY	TMPSTR	DY	EIQ	RIE	LGRC	IGEG	QFGDV
CHKFAK	401	IDEDTY	TMPSTR	DY	EIQ	RIE	LGRC	IGEG	QFGDV
HUMFAK	481	PHIVKL	IGVIT	ENP	WVIM	ELCT	LGEL	RSF	LQVR
MUSFAK	481	PHIVKL	IGVIT	ENP	WVIM	ELCT	LGEL	RSF	LQVR
CHKFAK	481	PHIVKL	IGVIT	ENP	WVIM	ELCT	LGEL	RSF	LQVR
HUMFAK	561	KLGD	FGLSR	YMED	STYYK	ASKG	KLPI	KWMA	PESIN
MUSFAK	561	KLGD	FGLSR	YMED	STYYK	ASKG	KLPI	KWMA	PESIN
CHKFAK	561	KLGD	FGLSR	YMED	STYYK	ASKG	KLPI	KWMA	PESIN

recombinant peptide

FALIAS_FAK2AS

kinase domain

FIG. 1A

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HUMFAK 641 LPMPNCPPTLYSLMTKCWAYDPSSRRPRFTTELKAQLSTILEEKAQOEERMESRRQATVSWDSGGSDEAPPKPSRPGY
MUSFAK 641 LPMPNCPPTLYSLMTKCWAYDPSSRRPRFTTELKAQLSTILEEKVQOEERMESRRQATVSWDSGGSDEAPPKPSRPGY
CHKFAK 641 LPMPNCPPTLYSLMTKCWAYDPSSRRPRFTTELKAQLSTILEEKLQOEERMESRRQVTVSNDSGGSDEAPPKPSRPGY

HUMFAK 721 PPSRSSEGFYPSPOHMQTNHYQVSGYPCSHGITAMAGSIYPGQASLLDQTDSDWNHRPOETAMWQPNVEDSTVLDLRGIG
MUSFAK 721 PPSRSSEGFYPSPOHMQTNHYQVSGYPCSHGITAMAGSIYQGOASLLDQTELMNHRPOEMSMWQPSVEDSAALDLRGMG
CHKFAK 721 PPSRSSEGFYPSPOHMQTNHYQVSGYPCSHGITAMAGSIYPGQASLLDQTDSDWNHRPOEVSQWQPNMEDSGTLDVRGGMG

HUMFAK 801 QVLPPTHLMEEERLIROQQEMEEDQRWLEKEERFL..KPDVRLSRGSIIDREDGSLQGPIGNQHIYQPVGKPDPAAPPKKPPR
MUSFAK 801 QVLPPTHLMEEERLIROQQEMEEDQRWLEKEERFL..KPDVRLSRGSIIDREDGSLQGPIGNQHIYQPVGKPDPAAPPKKPPR
CHKFAK 801 QVLPPTHLMEEERLIROQQEMEEDQRWLEKEERFLVMKPDVRLSRGSIIDREDGSLQGPIGNQHIYQPVGKPDPAAPPKKPPR

HUMFAK 879 PGAPGHLGSLASLSSPADSYNEGKVLQPOEISPPPTANLDRSNDKVYENVVTGLVKAVIEMSSKIQAPPEEYVPMVKEVG
MUSFAK 879 PGAPGHLGSLASLSSPADSYNEGKVLQPOEISPPPTANLDRSNDKVYENVVTGLVKAVIEMSSKIQAPPEEYVPMVKEVG
CHKFAK 881 PGAP.HLGSLASLNSPVDsYNEGKIKPQEISPPPTANLDRSNDKVYENVVTGLVKAVIEMSSKIQAPPEEYVPMVKEVG

HUMFAK 959 LALRTLATVDETIPLLPASTHREIEMAQKLLNSDLGELINKMKLAQQYVMTSLQQEYKKQMLTAAHALAVDAKNLLDVI
MUSFAK 959 LALRTLATVDETIPLLPASTHREIEMAQKLLNSDLGELISKMKAQQYVMTSLQQEYKKQMLTAAHALAVDAKNLLDVI
CHKFAK 960 LALRTLATVDESPLVLPASTHREIEMAQKLLNSDLAEELINKMKLAQQYVMTSLQQEYKKQMLTAAHALAVDAKNLLDVI

HUMFAK 1039 DQARLKMLGQTRPH
MUSFAK 1039 DQARLKMLGQTRPH
CHKFAK 1040 DQARLKMLGQTRPH

FIG. 1B

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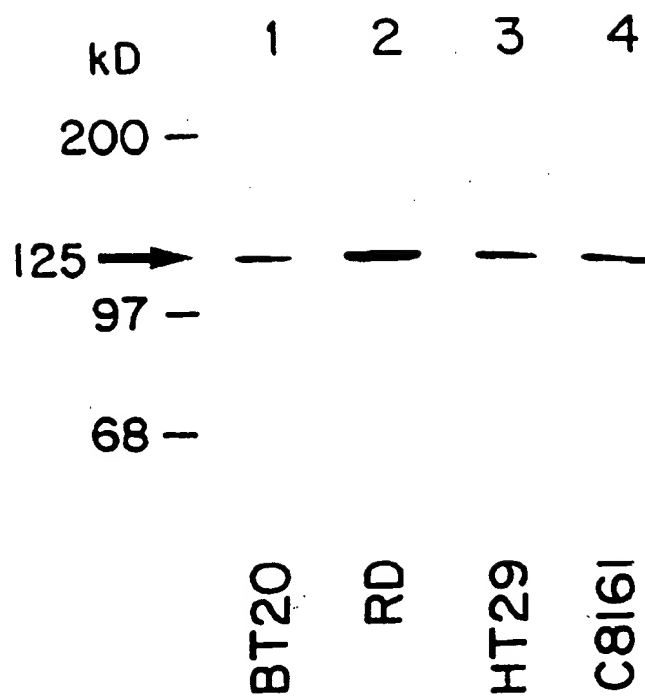


FIG. 2

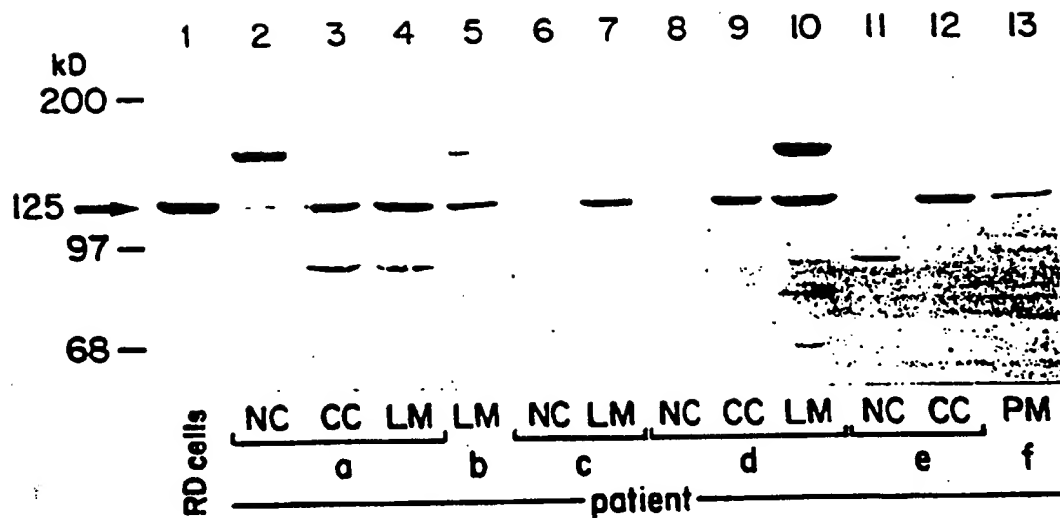


FIG. 3

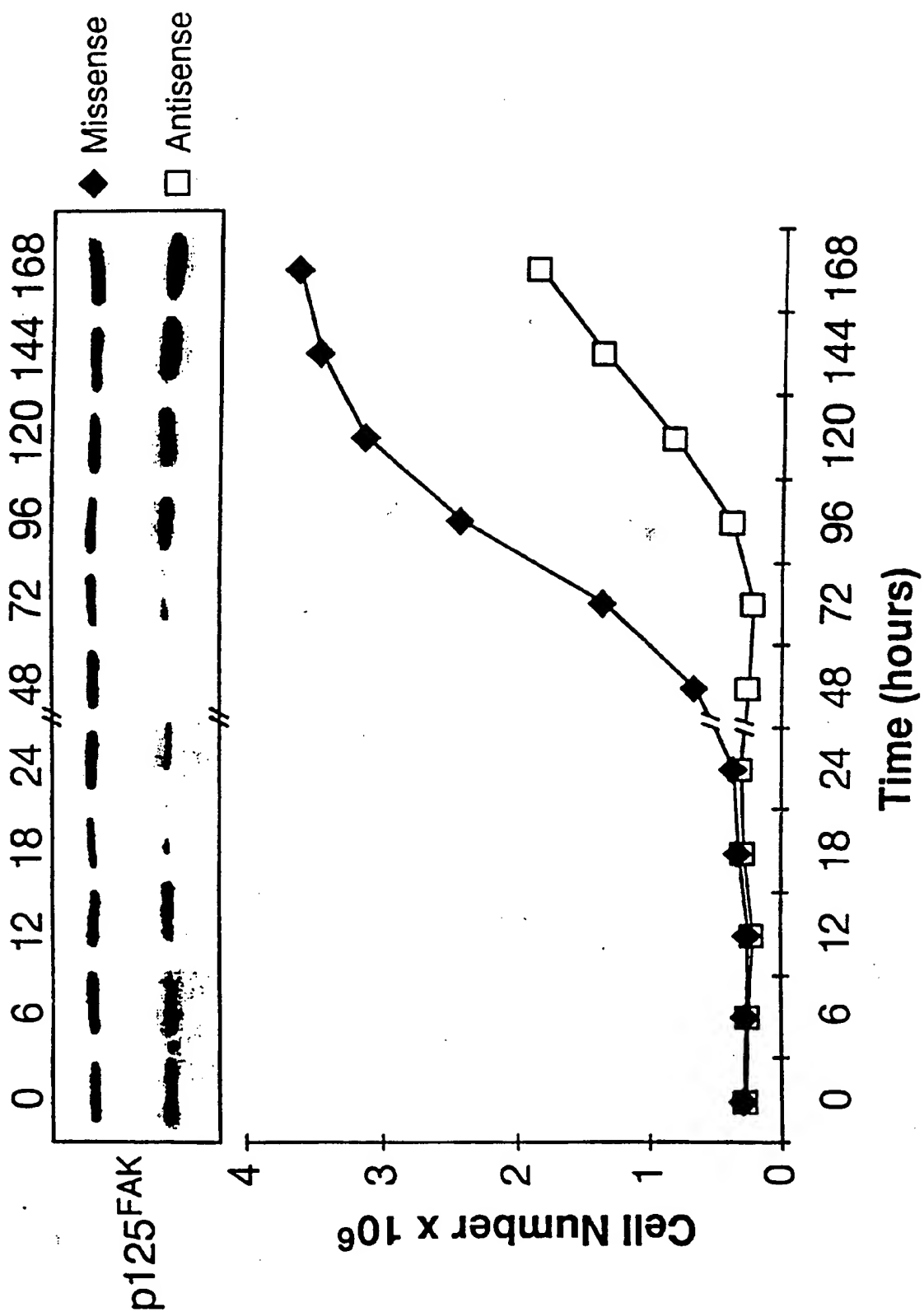


FIG. 4

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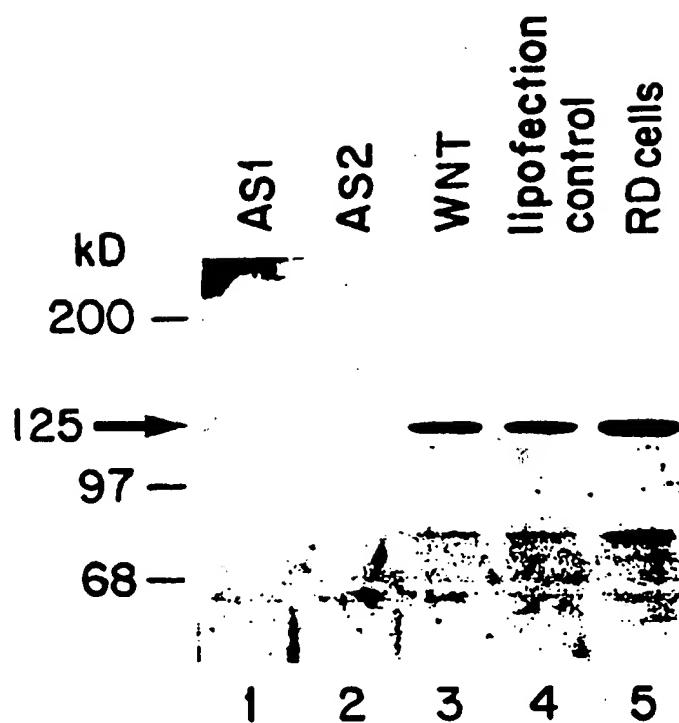


FIG. 5

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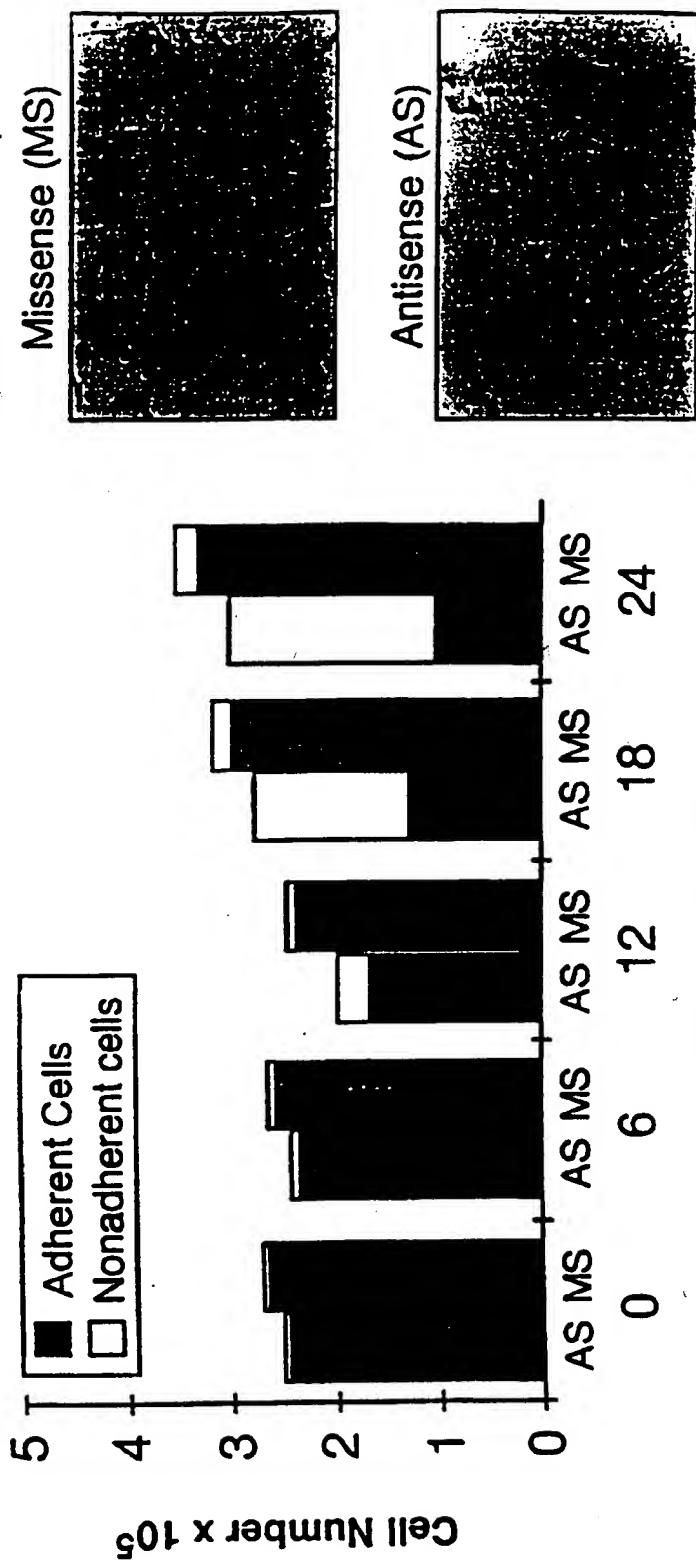


FIG. 6A

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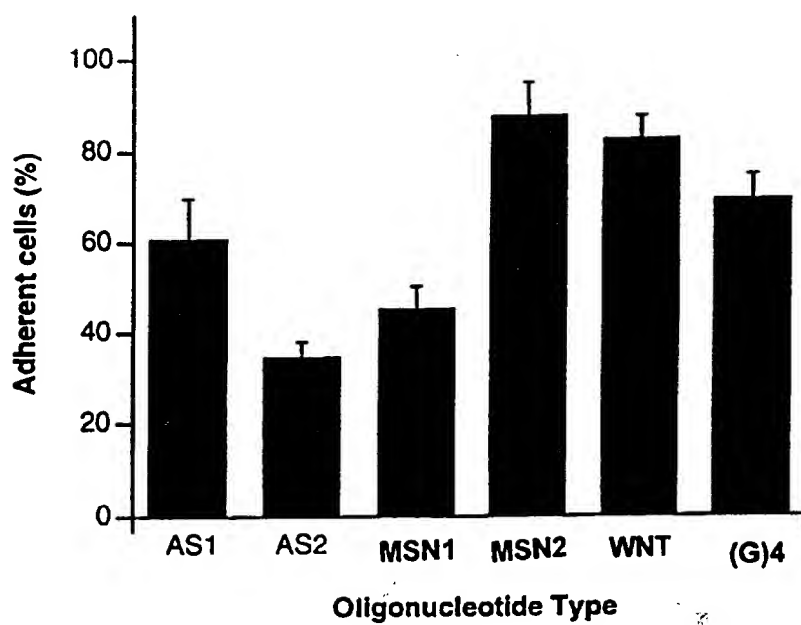


FIG. 6B

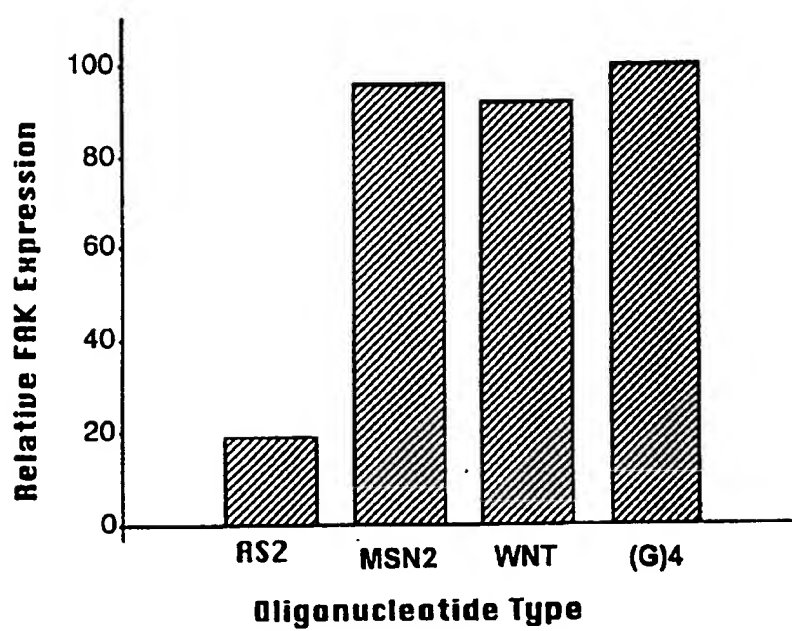


FIG. 6C

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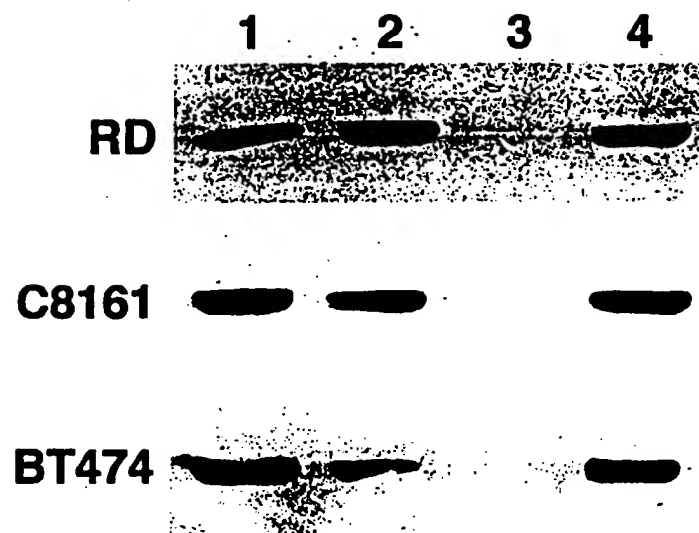
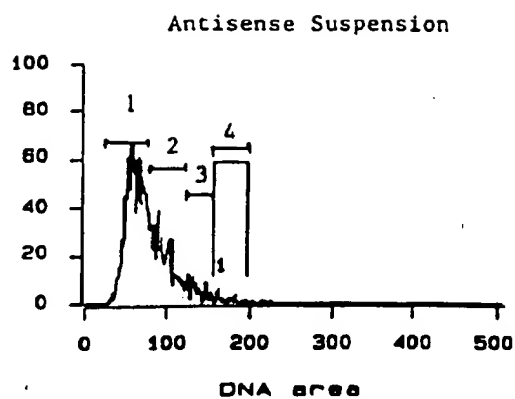


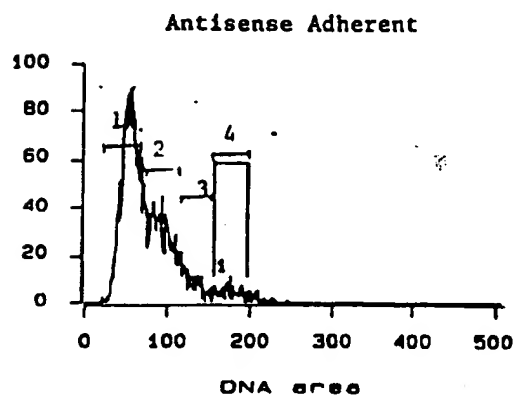
FIG. 7

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A.



B.



C.

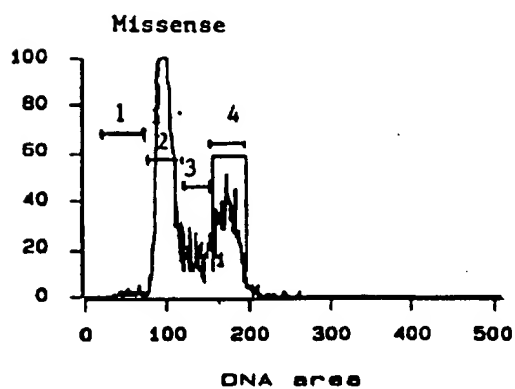


FIG. 8

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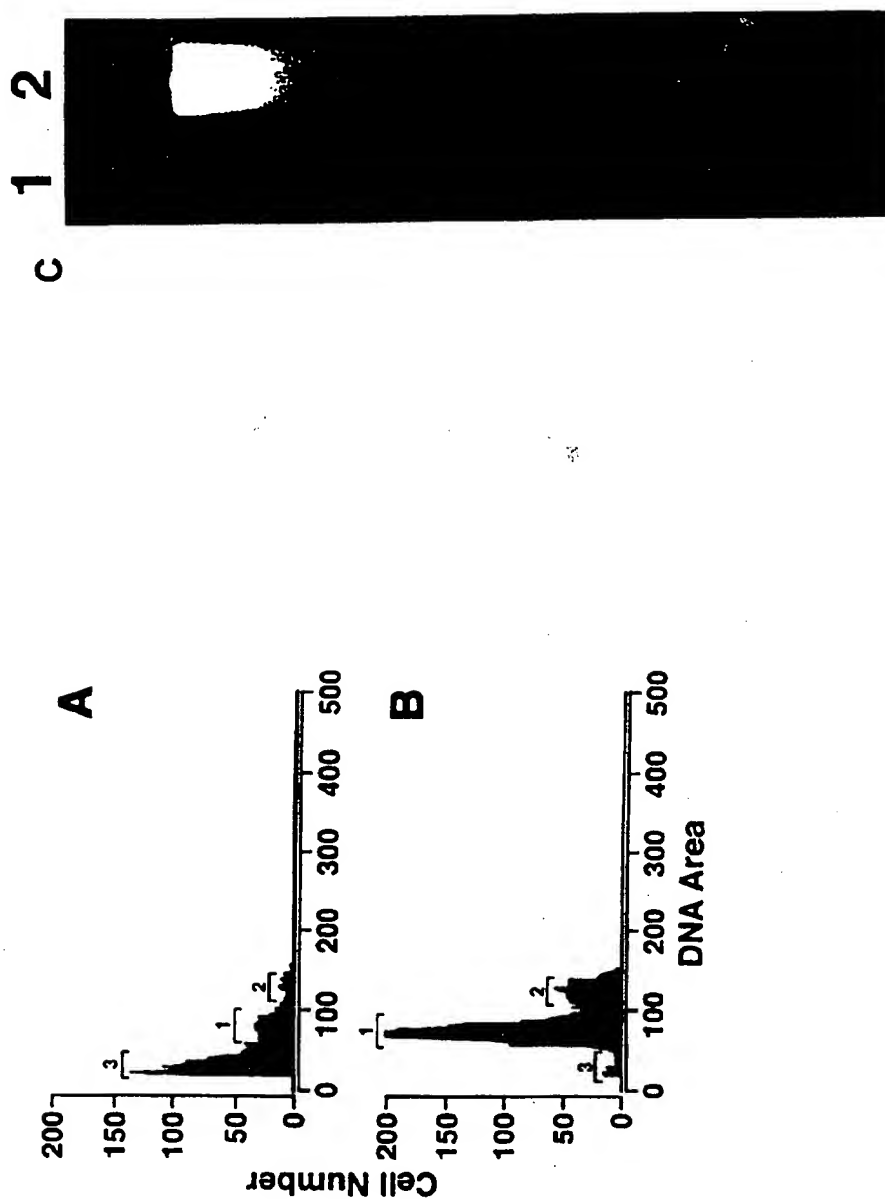


FIG. 9

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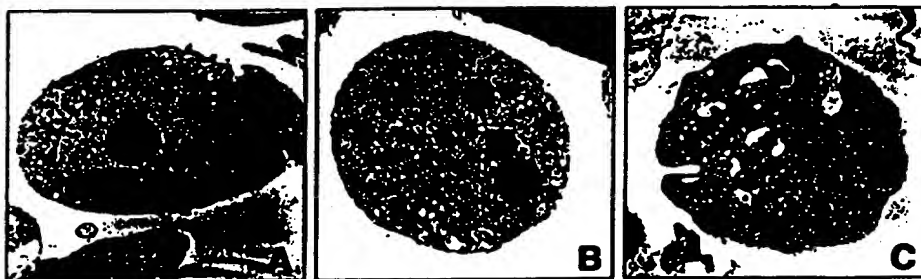


FIG. 10